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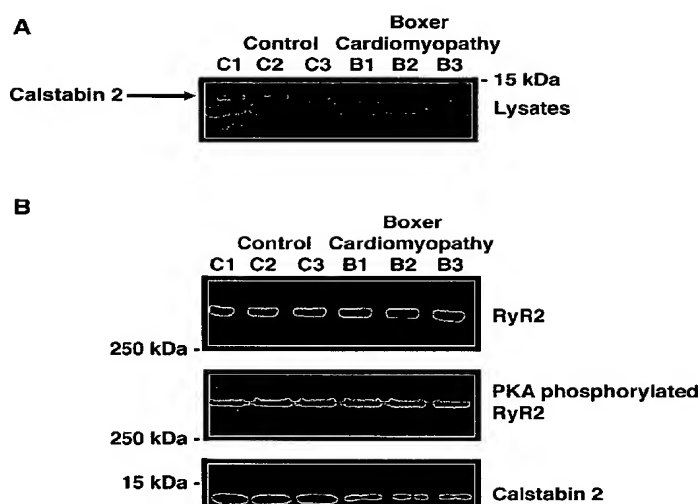
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(54) Title: METHODS AND COMPOSITIONS FOR TREATMENT OF CARDIAC ARRHYTHMIA IN NON-HUMAN ANIMALS



(57) Abstract: The present invention provides methods for detecting an arrhythmogenic condition in a subject non-human animal, such as a canine of the breed Boxer, comprising measuring a level of calstabin2 (FKBP 12.6) in cardiac tissue of the subject non-human animal, wherein the method measures and compares calstabin2 mRNA and/or levels of calstabin2 (FKBP 12.6) protein in the RyR2-calstabin2 protein complex in cardiac tissue, wherein a reduced level of calstabin2 (FKBP 12.6) is indicative of an arrhythmogenic condition. The present invention also provides methods for treating or preventing an arrhythmogenic condition in non-human animals, comprising administering an agent known to affect the interaction between calstabin2 and RyR2, wherein the agent is selected from the group of compounds of Formula (I) and Formula (II). The invention further provides animal feed compositions comprising compounds of Formula (I) and Formula (II).

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**METHODS AND COMPOSITIONS FOR TREATMENT OF CARDIAC
ARRHYTHMIA IN NON-HUMAN ANIMALS**

[0001] This application claims priority to USSN 60/795,205 filed on April 25, 2006 and international application PCT/US2006/032405 filed on August 17, 2006. The disclosures of these applications in their entirety are hereby incorporated by reference into this application.

[0002] This invention was made with government support under NHLBI PO1 HL 067849. As such the United States government has certain rights in this invention.

[0003] Throughout this application, various publications are referenced. The disclosures of these publications in their entirety are hereby incorporated by reference into this application in order to more fully describe the state of the art as known to those skilled therein as of the date of the invention described and claimed herein.

FIELD OF THE INVENTION

[0004] This invention is related to methods for diagnosing arrhythmogenic right ventricular cardiomyopathy (ARVC) in non-human animals, including canines of the breed Boxer. The invention further relates to methods for treating non-human animal subjects with ARVC by use of compounds which affect the ratio of calstabin to RyR in the RyR-calstabin protein complex.

BACKGROUND

[0005] Despite advances in treatment, congestive heart failure remains an important cause of mortality in Western countries. Heart failure affects 5 million individuals in the United States alone, and is characterized by a 5-year mortality rate of ~50% (Levy *et al.*, Long-term trends in the incidence of and survival with heart failure. *N. Engl. J. Med.*, 347:1397-402, 2002). It is notable that Sudden Cardiac Death (SCD) and disease conditions associated with heart failure are also observed in a number of non-human animals, including canines, felines, rodents, equines and so forth.

SUMMARY

[0006] In one aspect, the present invention is directed to a method for detecting a cardiac disorder or disease, which includes but is not limited to an arrhythmogenic condition in a

subject non-human animal, the a method comprising: (1) measuring a level of calstabin2 (FKBP12.6) in a cardiac tissue of a subject non-human animal, and (2) comparing the level of calstabin2 (FKBP12.6) in the cardiac tissue of the subject non-human animal to a level of calstabin2 (FKBP12.6) in a cardiac tissue of a control non-human animal, the control non-human animal known not to have an arrhythmogenic condition, wherein a reduced level of calstabin2 (FKBP12.6) in the cardiac tissue of the subject non-human animal compared to the level of calstabin2 (FKBP12.6) in the cardiac tissue of the control non-human animal indicates the presence of an arrhythmogenic condition. In certain embodiments, the calstabin2 is present in an RyR2-calstabin2 complex.

[0007] In another aspect, the present invention is directed to a method for detecting an arrhythmogenic condition in a subject non-human animal, the method comprising: (1) measuring a level of calstabin2 mRNA in cardiac tissue of the subject non-human animal; and, (2) comparing the level of calstabin2 mRNA in the cardiac tissue of the subject non-human animal to a level of calstabin2 mRNA in cardiac tissue of a control non-human animal, the control non-human animal known not to have an arrhythmogenic condition, wherein a reduced level of calstabin2 mRNA in the cardiac tissue of the subject non-human animal compared to the level of calstabin2 mRNA in the cardiac tissue of the control non-human animal indicates the presence of an arrhythmogenic condition. The levels of calstabin2 mRNA can be measured by a variety of methods known in the art including but no limited to Northern blot analysis, RT-PCR, real time RT-PCR.

[0008] In another aspect, the invention is directed to methods for detecting an arrhythmogenic condition in a subject non-human animal, comprising: (1) measuring a level of calstabin2 (FKBP12.6) in a cardiac tissue of the subject non-human animal, wherein the method measures protein levels of calstabin2 (FKBP12.6), and (2) comparing the level of calstabin2 (FKBP12.6) in the cardiac tissue of the subject non-human animal to a level of calstabin2 (FKBP12.6) in a cardiac tissue of a control non-human animal, the control non-human animal known not to have an arrhythmogenic condition, wherein a reduced level of calstabin2 (FKBP12.6) in the cardiac tissue of the subject non-human animal compared to the level of calstabin2 (FKBP12.6) in the cardiac tissue of the control non-human animal indicates the presence of a cardiac disorder or disease, including but not limited to an arrhythmogenic condition. In certain embodiments, the calstabin2 is present in an RyR2-calstabin complex.

[0009] In certain embodiments of the invention the non-human animal can be selected from the group consisting of canines, felines, equines, porcine, poultry, ruminants, and rodents. In non-limiting examples, the canine is selected from a breed of Boxer, German Shepherd, Miniature Schnauzer, West Highland White terrier, Dachshund, English Springer Spaniel, Golden Retriever, Doberman Pinscher, Newfoundland, Cocker Spaniel, Grate Dane, Irish Wolfhound, Afghan Hound, and Saluki. In non-limiting example, the feline is selected from the group of Maine coon cats. In non-limiting examples, the ruminant is selected from the group of sheep or cattle, including but not limited to cow. In non-limiting examples, the poultry is selected from the group of chicken, turkey, goose, and duck.

[0010] In certain embodiments, the methods of measuring calstabin 2 mRNA in cardiac tissue can be useful for diagnosis of an arrhythmogenic condition. In other embodiments, the methods for measuring levels of calstabin2 in a cardiac tissue, including but not limited to the levels of calstabin2 in an RyR2-calstabin protein complex from cardiac tissue, can be useful for diagnosis of an arrhythmogenic condition. In certain embodiments, the arrhythmogenic condition is selected from, but not limited to, the conditions from the group consisting of: sudden cardiac death, arrhythmogenic right ventricular cardiomyopathy, hypertrophic cardiomyopathy, dilated cardiomyopathy, restrictive cardiomyopathy, ventricular arrhythmias, sick sinus syndrome, atrial standstill, atrial fibrillation, sinus tachycardia, and ventricular fibrillations.

[0011] In another aspect, the methods of measuring calstabin 2 mRNA, or the methods of measuring levels of calstabin2 (FKBP12.6) in a cardiac tissue, or/and in the RyR2-calstabin protein complex from a cardiac tissue, can diagnose an arrhythmogenic condition, including but not limited to ARVC. In certain embodiments, the methods of measuring calstabin 2 mRNA, or the methods of measuring levels of calstabin2 (FKBP12.6) in the RyR2-calstabin protein complex are directed to a non-human animal.

[0012] In another aspect, the invention is directed to methods for detecting arrhythmogenic right ventricular cardiomyopathy (ARVC) in a subject canine, comprising: (1) measuring a level of calstabin2 mRNA in cardiac tissue of the subject canine; and, (2) comparing the level of calstabin2 mRNA in the cardiac tissue of the subject canine to a level of calstabin2 mRNA in cardiac tissue of a control canine, said control canine known not to have arrhythmogenic right ventricular cardiomyopathy; wherein a reduced level of calstabin2 mRNA in the cardiac tissue of the subject canine compared to the level of calstabin2 mRNA

in the cardiac tissue of the control canine indicates the presence of arrhythmogenic right ventricular cardiomyopathy.

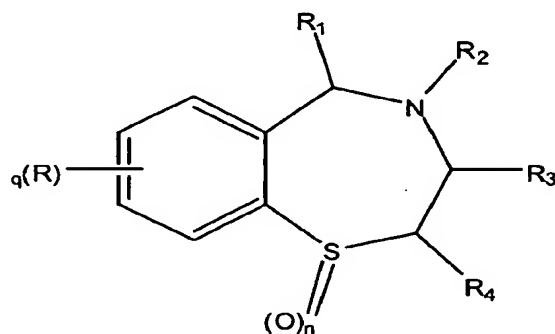
[0013] In certain embodiments of the methods of measuring calstabin 2 mRNA so as to diagnose an arrhythmogenic condition, including but not limited to ARVC, the reduced level of calstabin2 mRNA in the cardiac tissue of the subject non-human animal that indicates the presence of arrhythmogenic right ventricular cardiomyopathy is about ½ to about 1/20 the level of calstabin2 mRNA in the cardiac tissue of the control non-human animal.

[0014] In another aspect, the invention is directed to a method for detecting an arrhythmogenic right ventricular cardiomyopathy in a subject canine, comprising: (1) measuring a level of calstabin2 (FKBP12.6) in a cardiac tissue, including but not limited to measuring the level of calstabin2 in an RyR2-calstabin2 protein complex in a cardiac tissue of the subject canine, wherein the method measures protein levels of calstabin2 (FKBP12.6), and (2) comparing the level of calstabin2 (FKBP12.6) in the RyR2-calstabin2 protein complex in the cardiac tissue of the subject canine to a level of calstabin2 (FKBP12.6) in an RyR2-calstabin2 protein complex in cardiac tissue of a control canine, the control canine known not to have an arrhythmogenic right ventricular cardiomyopathy, wherein a reduced level of calstabin2 (FKBP12.6) in the RyR2-calstabin2 protein complex in the cardiac tissue of the subject canine compared to the level of calstabin2 (FKBP12.6) in the RyR2-calstabin2 protein complex in the cardiac tissue of the control canine indicates the presence of an arrhythmogenic right ventricular cardiomyopathy.

[0015] In certain embodiments, the reduced level of calstabin2 (FKBP12.6) in the cardiac tissue of the subject non-human animal indicates the presence of a cardiac disorder or disease. In certain embodiments, the disorder or disease is an arrhythmogenic condition, which includes but is not limited to arrhythmogenic right ventricular cardiomyopathy. In certain embodiments, the reduced level of calstabin2 (FKBP12.6) in the RyR2-calstabin2 protein complex in the cardiac tissue of the subject non-human animal indicates the presence of an arrhythmogenic condition, which includes but is not limited to arrhythmogenic right ventricular cardiomyopathy. In certain embodiments, the reduced level of calstabin2 (FKBP12.6) in the RyR2-calstabin2 protein complex in the cardiac tissue of the subject non-human animal, which in a non-limiting example is a canine, can be reduced by about 2 times to about a level which is below the threshold of detection

compared to the level of calstabin2 (FKBP12.6) in the RyR2-calstabin2 complex in the cardiac tissue of the control canine. The levels of calstabin2 in the in the RyR2-calstabin2 protein complex can be measured by any suitable method known in the art.

[0016] In one aspect, the present invention is directed to a method for treating a disorder or disease in a non-human subject animal, which is or is suspect to be in need thereof, the method comprising administering to the non-human animal an agent known to affect the interaction of the calstabin protein to a RyR receptor in a RyR-calstabin protein complex. In certain embodiments, the calstabin protein is calstabin2 and the RyR protein is RyR2. The present invention is directed to a method for treating a disorder or disease in a non-human animal, wherein the disorder or disease can be any cardiac disorder or disease, including but not limited to an arrhythmogenic condition, the method comprising administering to the non-human animal an agent known to affect the interaction of the calstabin protein to the RyR receptor in the RyR-calstabin protein complex. In certain embodiments, the agent is selected from the group of compounds of the general Formula I:



wherein,

n is 0, 1, or 2;

q is 0, 1, 2, 3, or 4;

each R is independently selected from the group consisting of H, halogen, -OH, -NH₂, -NO₂, -CN, -CF₃, -OCF₃, -N₃, -SO₃H, -S(=O)₂alkyl, -S(=O)alkyl, -OS(=O)₂CF₃, acyl, -O-acyl, alkyl, alkoxy, alkylamino, alkylarylamino, alkylthio, cycloalkyl, alkylaryl, aryl, heteroaryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino; wherein each acyl, -O-acyl, alkyl, alkoxy, alkylamino, alkylarylamino, alkylthio, cycloalkyl, alkylaryl, aryl, heteroaryl, heterocyclyl,

heterocyclalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino may be optionally substituted;

R₁ is selected from the group consisting of H, oxo, alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocycl; wherein each alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocycl may be optionally substituted;

R₂ is selected from the group consisting of H, -C(=O)R₅, -C(=S)R₆, -SO₂R₇, -P(=O)R₈R₉, -(CH₂)_m-R₁₀, alkyl, aryl, alkylaryl, heteroaryl, cycloalkyl, cycloalkylalkyl, and heterocycl; wherein each alkyl, aryl, alkylaryl, heteroaryl, cycloalkyl, cycloalkylalkyl, and heterocycl may be optionally substituted;

R₃ is selected from the group consisting of H, -CO₂Y, -C(=O)NHY, acyl, -O-acyl, alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocycl; wherein each acyl, alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocycl may be optionally substituted; and wherein Y is selected from the group consisting of H, alkyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocycl, and wherein each alkyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocycl may be optionally substituted;

R₄ is selected from the group consisting of H, alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocycl; wherein each alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocycl may be optionally substituted;

R₅ is selected from the group consisting of -NR₁₅R₁₆, -(CH₂)_qNR₁₅R₁₆, -NHNHNR₁₅R₁₆, -NHOH, -OR₁₅, -C(=O)NHNHNR₁₅R₁₆, -CO₂R₁₅, -C(=O)NR₁₅R₁₆, -CH₂X, acyl, alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocycl, and heterocyclalkyl; wherein each acyl, alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocycl, and heterocyclalkyl may be optionally substituted, and wherein q is 1, 2, 3, 4, 5, or 6;

R₆ is selected from the group consisting of -OR₁₅, -NHNHNR₁₅R₁₆, -NHOH, -NR₁₅R₁₆, -CH₂X, acyl, alkenyl, alkyl, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocycl, and heterocyclalkyl; wherein each acyl, alkenyl, alkyl, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocycl, and heterocyclalkyl may be optionally substituted;

R₇ is selected from the group consisting of -OR₁₅, -NR₁₅R₁₆, -NHNHNR₁₅R₁₆, -NHOH, -CH₂X, alkyl, alkenyl, alkynyl, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocycl, and heterocyclalkyl; wherein each alkyl, alkenyl, alkynyl, aryl, alkylaryl, cycloalkyl,

cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclalkyl may be optionally substituted;

R_8 and R_9 independently are selected from the group consisting of OH, acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclalkyl; wherein each acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclalkyl may be optionally substituted;

R_{10} is selected from the group consisting of $-NR_{15}R_{16}$, OH, $-SO_2R_{11}$, $-NHSO_2R_{11}$, $C(=O)(R_{12})$, $NHC(=O)(R_{12})$, $-OC(=O)(R_{12})$, and $-P(=O)R_{13}R_{14}$;

R_{11} , R_{12} , R_{13} , and R_{14} independently are selected from the group consisting of H, OH, NH_2 , $-NHNH_2$, $-NHOH$, acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclalkyl; wherein each acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclalkyl may be optionally substituted;

X is selected from the group consisting of halogen, $-CN$, $-CO_2R_{15}$, $-C(=O)NR_{15}R_{16}$, $-NR_{15}R_{16}$, $-OR_{15}$, $-SO_2R_7$, and $-P(=O)R_8R_9$; and

R_{15} and R_{16} independently are selected from the group consisting of H, acyl, alkenyl, alkoxyl, OH, NH_2 , alkyl, alkylamino, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclalkyl; wherein each acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclalkyl may be optionally substituted; and optionally R_{15} and R_{16} together with the N to which they are bonded may form a heterocycle which may be substituted;

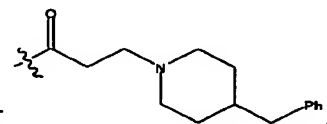
the nitrogen in the benzothiazepine ring may optionally be a quaternary nitrogen; and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, and prodrugs thereof;

provided that when q is 0 and n is 0, then R_2 is not H, Et, $-C(=O)NH_2$, $(=O)NHPh$, $-C(=S)NH-nButyl$, $-C(=O)NHC(=O)CH_2Cl$, $-C(=O)H$, $-C(=O)Me$, $-C(=O)Et$, $-C(=O)CH=CH_2$, $-S(=O)_2Me$, or $-S(=O)_2Et$;

further provided that when q is 0 and n is 1 or 2, then R_2 is not $-C(=O)Me$, $-C(=O)Et$, $-S(=O)_2Me$, or $-S(=O)_2Et$;

further provided that when q is 1, and R is Me, Cl, or F at the 6 position of the benzothiazepene ring, then R_2 is not H, Me, $-\text{C}(=\text{O})\text{H}$, $-\text{C}(=\text{O})\text{Me}$, $-\text{C}(=\text{O})\text{Et}$, $-\text{C}(=\text{O})\text{Ph}$, $-\text{S}(=\text{O})_2\text{Me}$, or $-\text{S}(=\text{O})_2\text{Et}$; and

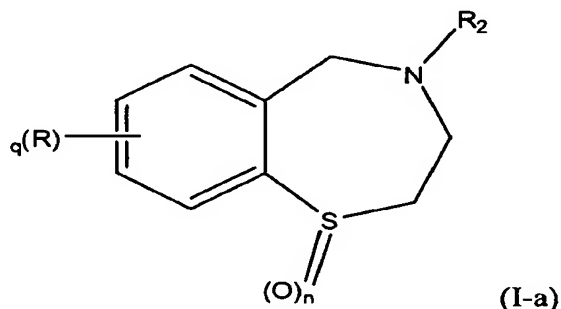
further provided that when q is 1, n is 0, and R is OCT_3 , OH, $\text{C}_1\text{-C}_3$ alkoxyl at the 7 position



of the benzothiazepene ring, then R_2 is not H, $-\text{C}(=\text{O})\text{CH}=\text{CH}_2$, or

[0017] In one embodiment, the present invention provides compounds of Formula I, as described above, with the proviso that the compound is not S24 or S68.

[0018] In one embodiment, the methods of the present invention comprise administering to a subject a compound of Formula I-a, or enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, or prodrugs, or a combination thereof:



wherein:

n is 0, 1, or 2;

q is 0, 1, 2, 3, or 4;

each R is independently selected from the group consisting of H, halogen, $-\text{OH}$, $-\text{NH}_2$, $-\text{NO}_2$, $-\text{CN}$, $-\text{CF}_3$, $-\text{OCF}_3$, $-\text{N}_3$, $-\text{SO}_3\text{H}$, $-\text{S}(=\text{O})_2\text{alkyl}$, $-\text{S}(=\text{O})\text{alkyl}$, $-\text{OS}(=\text{O})_2\text{CF}_3$, acyl, alkyl, alkoxyl, alkylamino, alkylthio, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)aryl amino; wherein each acyl, alkyl, alkoxyl, alkylamino, alkylthio, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)aryl amino may be substituted or unsubstituted;

R_2 is selected from the group consisting of H, $-\text{C}=\text{O}(\text{R}_5)$, $-\text{C}=\text{S}(\text{R}_6)$, $-\text{SO}_2\text{R}_7$, $-\text{P}(=\text{O})\text{R}_8\text{R}_9$, $-(\text{CH}_2)_m-\text{R}_{10}$, alkyl, aryl, heteroaryl, cycloalkyl, cycloalkylalkyl, and heterocyclyl; wherein each alkyl, aryl, heteroaryl, cycloalkyl, cycloalkylalkyl, and heterocyclyl may be substituted or unsubstituted;

R_5 is selected from the group consisting of $-\text{NR}_{15}\text{R}_{16}$, $-\text{NHN}\text{R}_{15}\text{R}_{16}$, $-\text{NHOH}$, $-\text{OR}_{15}$, $-\text{C}(=\text{O})\text{NHN}\text{R}_{15}\text{R}_{16}$, $-\text{CO}_2\text{R}_{15}$, $-\text{C}(=\text{O})\text{NR}_{15}\text{R}_{16}$, $-\text{CH}_2\text{X}$, acyl, alkyl, alkenyl, alkynyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkyl, alkenyl, alkynyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted;

R_6 is selected from the group consisting of $-\text{OR}_{15}$, $-\text{NHN}\text{R}_{15}\text{R}_{16}$, $-\text{NHOH}$, $-\text{NR}_{15}\text{R}_{16}$, $-\text{CH}_2\text{X}$, acyl, alkenyl, alkyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkenyl, alkyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted;

R_7 is selected from the group consisting of H, $-\text{OR}_{15}$, $-\text{NR}_{15}\text{R}_{16}$, $-\text{NHN}\text{R}_{15}\text{R}_{16}$, $-\text{NHOH}$, $-\text{CH}_2\text{X}$, alkyl, alkenyl, alkynyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each alkyl, alkenyl, alkynyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted;

R_8 and R_9 independently are selected from the group consisting of $-\text{OH}$, acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted;

R_{10} is selected from the group consisting of $-\text{NR}_{15}\text{R}_{16}$, OH , $-\text{SO}_2\text{R}_{11}$, $-\text{NHSO}_2\text{R}_{11}$, $-\text{C}(=\text{O})\text{R}_{12}$, $-\text{NH}(\text{C}=\text{O})\text{R}_{12}$, $-\text{O}(\text{C}=\text{O})\text{R}_{12}$, and $-\text{P}(=\text{O})\text{R}_{13}\text{R}_{14}$;

m is 0, 1, 2, 3, or 4;

R_{11} , R_{12} , R_{13} , and R_{14} independently are selected from the group consisting of H, OH, NH_2 , $-\text{NHNH}_2$, $-\text{NHOH}$, acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted;

X is selected from the group consisting of halogen, $-\text{CN}$, $-\text{CO}_2\text{R}_{15}$, $-\text{C}(=\text{O})\text{NR}_{15}\text{R}_{16}$, $-\text{NR}_{15}\text{R}_{16}$, $-\text{OR}_{15}$, $-\text{SO}_2\text{R}_7$, and $-\text{P}(=\text{O})\text{R}_8\text{R}_9$; and

R_{15} and R_{16} independently are selected from the group consisting of H, acyl, alkenyl, alkoxy, OH, NH_2 , alkyl, alkylamino, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclalkyl; wherein each acyl, alkenyl, alkoxy, alkyl, alkylamino, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclalkyl may be substituted or unsubstituted; and optionally R_{15} and R_{16} together with the N to which they are bonded may form a heterocycle which may be substituted or unsubstituted;

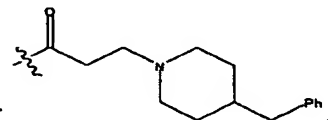
the nitrogen in the benzothiazepine ring may be optionally a quaternary nitrogen; and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, and prodrugs thereof;

provided that when q is 0 and n is 0, then R_2 is not H, Et, $-C(=O)NH_2$, $(=O)NHPH$, $-C(=S)NH-nButyl$, $-C(=O)NHC(=O)CH_2Cl$, $-C(=O)H$, $-C(=O)Me$, $-C(=O)Et$, $-C(=O)CH=CH_2$, $-S(=O)_2Me$, or $-S(=O)_2Et$;

further provided that when q is 0 and n is 1 or 2, then R_2 is not $-C(=O)Me$, $-C(=O)Et$, $-S(=O)_2Me$, or $-S(=O)_2Et$;

further provided that when q is 1, and R is Me, Cl, or F at the 6 position of the benzothiazepene ring, then R_2 is not H, Me, $-C(=O)H$, $-C(=O)Me$, $-C(=O)Et$, $-C(=O)Ph$, $-S(=O)_2Me$, or $-S(=O)_2Et$; and

further provided that when q is 1, n is 0, and R is OCT_3 , OH, C_1-C_3 alkoxy at the 7 position



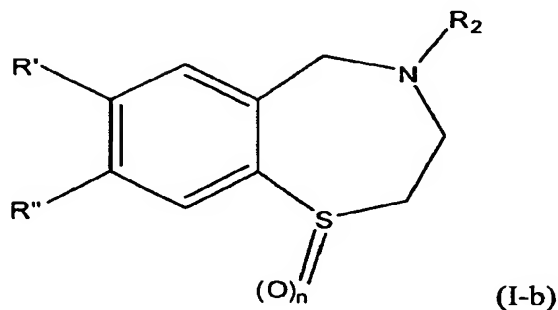
of the benzothiazepene ring, then R_2 is not H, $-C(=O)CH=CH_2$, or

[0019] In certain embodiments, the present invention provides compounds of formula **I-a**, wherein each R is independently selected from the group consisting of H, halogen, $-OH$, OMe , $-NH_2$, $-NO_2$, $-CN$, $-CF_3$, $-OCF_3$, $-N_3$, $-S(=O)_2C_1-C_4alkyl$, $-S(=O)C_1-C_4alkyl$, $-S-C_1-C_4alkyl$, $-OS(=O)_2CF_3$, Ph, $-NHCH_2Ph$, $-C(=O)Me$, $-OC(=O)Me$, morpholinyl and propenyl; and n is 0, 1, or 2.

[0020] In other embodiments, the present invention provides compounds of formula **I-a**, wherein R_2 is selected from the group consisting of $-C(=O)(R_5)$, $-C(=S)(R_6)$, $-SO_2R_7$, $-P(=O)R_8R_9$, and $-(CH_2)_m-R_{10}$.

[0021] In one embodiment, the methods of the present invention comprise administering to a subject a compound of Formula **I-b**, or enantiomers, diastereomers, tautomers,

pharmaceutically acceptable salts, hydrates, solvates, complexes, or prodrugs, or a combination thereof:



wherein R' and R'' are independently selected from the group consisting of H, halogen, -OH, -NH₂, -NO₂, -CN, -CF₃, -OCF₃, -N₃, -SO₃H, -S(=O)₂alkyl, -S(=O)alkyl, -OS(=O)₂CF₃, acyl, alkyl, alkoxyl, alkylamino, alkylthio, cycloalkyl, aryl, heterocyclyl, heterocyclalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylmino; and wherein each acyl, alkyl, alkoxyl, alkylamino, cycloalkyl, aryl, heterocyclyl, heterocyclalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio may be substituted or unsubstituted;

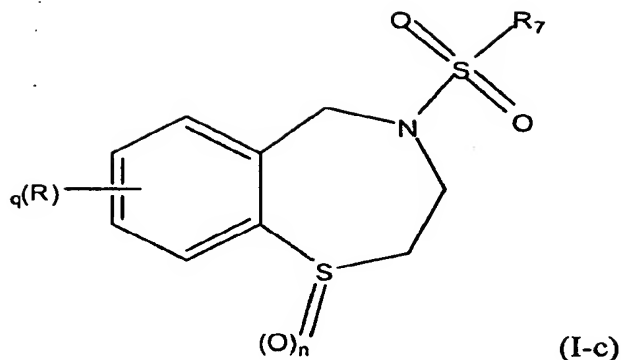
R₂ and n are as defined in compounds of formula I-a above;

and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

[0022] In certain embodiments, the present invention provides compounds of formula I-b, wherein R' and R'' are independently selected from the group consisting of H, halogen, -OH, OMe, -NH₂, -NO₂, -CN, -CF₃, -OCF₃, -N₃, -S(=O)₂C₁-C₄alkyl, -S(=O)C₁-C₄alkyl, -S-C₁-C₄alkyl, -OS(=O)₂CF₃, Ph, -NHCH₂Ph, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1 or 3. In some cases, R' is H or OMe, and R'' is H.

[0023] In other embodiments, the present invention provides compounds of formula I-b, wherein R₂ is selected from the group consisting of -C=O(R₅), -C=S(R₆), -SO₂R₇, -P(=O)R₈R₉, and -(CH₂)_m-R₁₀.

[0024] In yet another embodiment, the methods of the present invention comprise administering to a subject a compound of Formula I-c, or enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, or prodrugs, or a combination thereof:

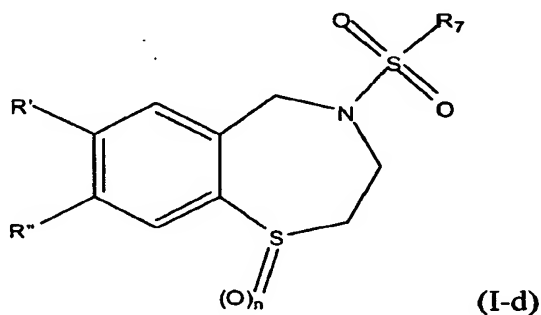


wherein each R, R₇, q, and n is as defined in compounds of formula I-a above; and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

[0025] In certain embodiments, the present invention provides compounds of formula I-c, wherein each R is independently selected from the group consisting of H, halogen, -OH, OMe, -NH₂, -NO₂, -CN, -CF₃, -OCF₃, -N₃, -S(=O)₂C₁-C₄alkyl, -S(=O)C₁-C₄alkyl, -S-C₁-C₄alkyl, -OS(=O)₂CF₃, Ph, -NHCH₂Ph, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1, or 2.

[0026] In other embodiments, the present invention provides compounds of formula I-c, wherein R₇ is selected from the group consisting of -OH, -NR₁₅R₁₆, alkyl, alkenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each alkyl, alkenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted.

[0027] In a further embodiment, the methods of the present invention comprise administering to a subject a compound of Formula I-d, or enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, or prodrugs, or a combination thereof:



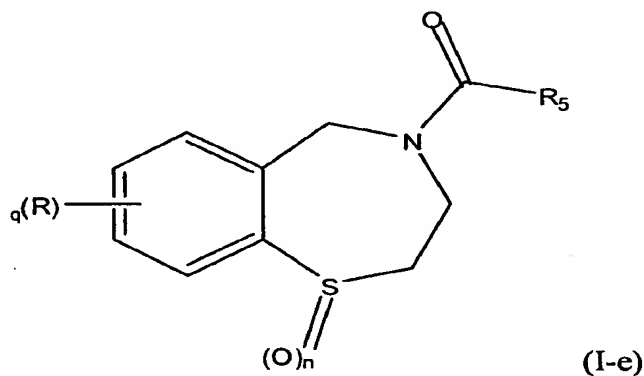
wherein R' and R'' are independently selected from the group consisting of H, halogen, -OH, -NH₂, -NO₂, -CN, -CF₃, -OCF₃, -N₃, -SO₃H, -S(=O)₂alkyl, -S(=O)alkyl, -OS(=O)₂CF₃, acyl, alkyl, alkoxy, alkylamino, alkylthio, cycloalkyl, aryl, heterocyclyl, heterocyclalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino; and wherein each acyl, alkyl, alkoxy, alkylamino, cycloalkyl, aryl, heterocyclyl, heterocyclalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio may be substituted or unsubstituted;

R₇ and n are as defined in compounds of formula I-a above; and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

[0028] In certain embodiments, the present invention provides compounds of formula I-d, wherein R' and R'' are independently selected from the group consisting of H, halogen, -OH, OMe, -NH₂, -NO₂, -CN, -CF₃, -OCF₃, -N₃, -S(=O)₂C₁-C₄alkyl, -S(=O)C₁-C₄alkyl, -SC₁-C₄alkyl, -OS(=O)₂CF₃, Ph, -NHCH₂Ph, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1 or 3. In some cases, R' is H or OMe, and R'' is H.

[0029] In other embodiments, the present invention provides compounds of formula I-d, wherein R₇ is selected from the group consisting of -OH, -NR₁₅R₁₆, alkyl, alkenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclalkyl; wherein each alkyl, alkenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclalkyl may be substituted or unsubstituted.

[0030] In one embodiment, the methods of the present invention comprise administering to a subject a compound of Formula I-e, or enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, or prodrugs, or a combination thereof:



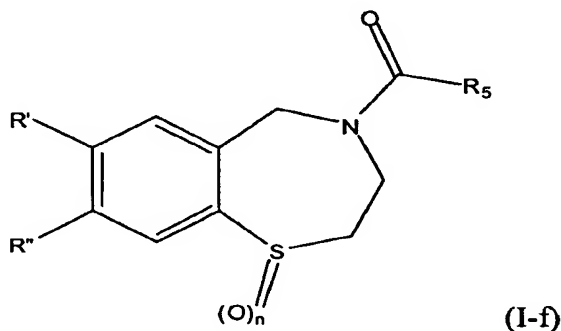
wherein each R, R₅, q and n is as defined compounds of formula I-a above; and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

[0031] In certain embodiments, the present invention provides compounds of formula I-e, wherein each R is independently selected from the group consisting of H, halogen, -OH, OMe, -NH₂, -NO₂, -CN, -CF₃, -OCF₃, -N₃, -S(=O)₂C₁-C₄alkyl, -S(=O)C₁-C₄alkyl, -S-C₁-C₄alkyl, -OS(=O)₂CF₃, Ph, -NHCH₂Ph, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1, or 2.

[0032] In other embodiments, the present invention provides compounds of formula I-e, wherein R₅ is selected from the group consisting of -NR₁₅R₁₆, -NHOH, -OR₁₅, -CH₂X, alkyl, alkenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkyl, alkenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted.

[0033] In some embodiments, the present invention provides compounds of formula I-e, wherein R₅ is an alkyl substituted by at least one labeling group, such as a fluorescent, a bioluminescent, a chemiluminescent, a colorimetric and a radioactive labeling group. A fluorescent labeling group can be selected from bodipy, dansyl, fluorescein, rhodamine, Texas red, cyanine dyes, pyrene, coumarins, Cascade BlueTM, Pacific Blue, Marina Blue, Oregon Green, 4',6-Diamidino-2-phenylindole (DAPI), indopyra dyes, lucifer yellow, propidium iodide, porphyrins, arginine, and variants and derivatives thereof.

[0034] In another embodiment, the methods of the present invention comprise administering to a subject a compound of Formula I-f, or enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, or prodrugs, or a combination thereof:



wherein R' and R'' are independently selected from the group consisting of H, halogen, -OH, -NH₂, -NO₂, -CN, -CF₃, -OCF₃, -N₃, -SO₃H, -S(=O)₂alkyl, -S(=O)alkyl, -OS(=O)₂CF₃, acyl, alkyl, alkoxyl, alkylamino, alkylthio, cycloalkyl, aryl, heterocyclyl, heterocyclalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylmino; and wherein each acyl, alkyl, alkoxyl, alkylamino, cycloalkyl, aryl, heterocyclyl, heterocyclalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio may be substituted or unsubstituted;

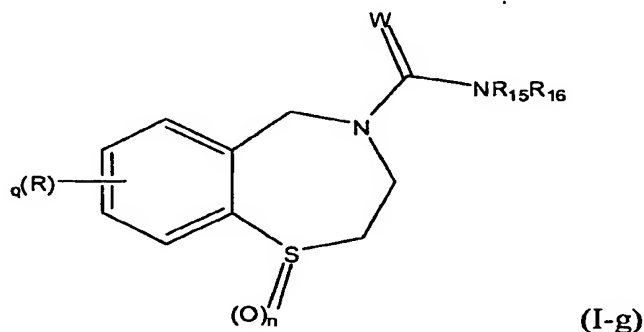
R₅ and n are as defined in compounds of formula I-a above;

and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

[0035] In certain embodiments, the present invention provides compounds of formula I-f, wherein R' and R'' are independently selected from the group consisting of H, halogen, -OH, OMe, -NH₂, -NO₂, -CN, -CF₃, -OCF₃, -N₃, -S(=O)₂C₁-C₄alkyl, -S(=O)C₁-C₄alkyl, -S-C₁-C₄alkyl, -OS(=O)₂CF₃, Ph, -NHCH₂Ph, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1 or 3. In some cases, R' is H or OMe, and R'' is H.

[0036] In other embodiments, the present invention provides compounds of formula I-f, wherein R₅ is selected from the group consisting of -NR₁₅R₁₆, -NHOH, -OR₁₅, -CH₂X, alkyl, alkenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclalkyl; wherein each acyl, alkyl, alkenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclalkyl may be substituted or unsubstituted.

[0037] In yet another embodiment, the methods of the present invention comprise administering to a subject a compound of Formula I-g, or enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, or prodrugs, or a combination thereof:



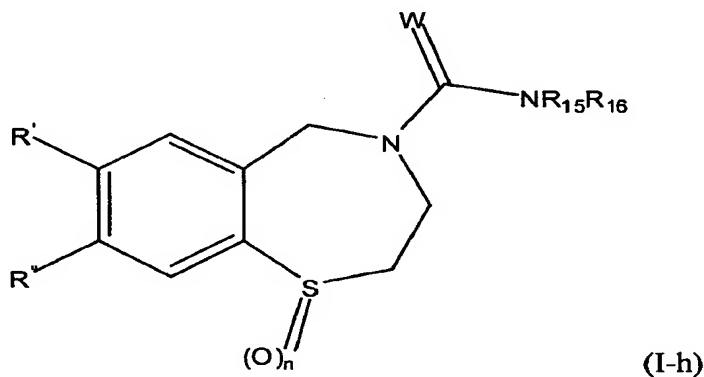
wherein W is S or O; each R, R₁₅, R₁₆, q, and n is as defined in compounds of formula I-a above; and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

[0038] In certain embodiments, the present invention provides compounds of formula I-g, wherein each R is independently selected from the group consisting of H, halogen, -OH, OMe, -NH₂, -NO₂, -CN, -CF₃, -OCF₃, -N₃, -S(=O)₂C₁-C₄alkyl, -S(=O)C₁-C₄alkyl, -S-C₁-C₄alkyl, -OS(=O)₂CF₃, Ph, -NHCH₂Ph, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1, or 2.

[0039] In other embodiments, the present invention provides compounds of formula I-g, wherein R₁₅ and R₁₆ independently are selected from the group consisting of H, OH, NH₂, alkyl, alkylamino, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each alkyl, alkylamino, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted; and optionally R₁₅ and R₁₆ together with the N to which they are bonded may form a heterocycle which may be substituted.

[0040] In some embodiments, the present invention provides compounds of formula I-g, wherein W is O or S.

[0041] In yet another embodiment, the methods of the present invention comprise administering to a subject a compound of Formula I-h, or enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, or prodrugs, or a combination thereof:



wherein W is S or O;

wherein R' and R'' are independently selected from the group consisting of H, halogen, -OH, -NH₂, -NO₂, -CN, -CF₃, -OCF₃, -N₃, -SO₃H, -S(=O)₂alkyl, -S(=O)alkyl, -OS(=O)₂CF₃, acyl, alkyl, alkoxy, alkylamino, alkylthio, cycloalkyl, aryl, heterocyclyl, heterocyclalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino; and wherein each acyl, alkyl, alkoxy, alkylamino, cycloalkyl, aryl, heterocyclyl, heterocyclalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio may be substituted or unsubstituted;

R₁₅, R₁₆ and n are as defined in compounds of formula **I-a** above;

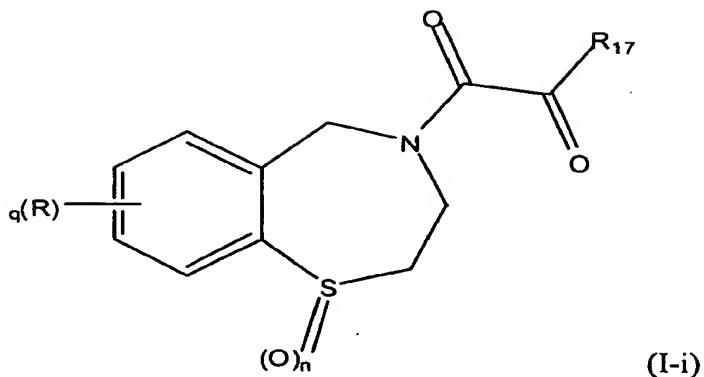
and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

[0042] In certain embodiments, the present invention provides compounds of formula **I-h**, wherein R' and R'' are independently selected from the group consisting of H, halogen, -OH, OMe, -NH₂, -NO₂, -CN, -CF₃, -OCF₃, -N₃, -S(=O)₂C₁-C₄alkyl, -S(=O)C₁-C₄alkyl, -S-C₁-C₄alkyl, -OS(=O)₂CF₃, Ph, -NHCH₂Ph, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1 or 3. In some cases, R' is H or OMe, and R'' is H.

[0043] In other embodiments, the present invention provides compounds of formula **I-h**, wherein R₁₅ and R₁₆ independently are selected from the group consisting of H, OH, NH₂, alkyl, alkylamino, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclalkyl; wherein each alkyl, alkylamino, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclalkyl may be substituted; and optionally R₁₅ and R₁₆ together with the N to which they are bonded may form a heterocycle which may be substituted.

[0044] In some embodiments, the present invention provides compounds of formula **I-g**, wherein W is O or S.

[0045] In a further embodiment, the methods of the present invention comprise administering to a subject a compound of Formula **I-i**, or enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, or prodrugs, or a combination thereof:



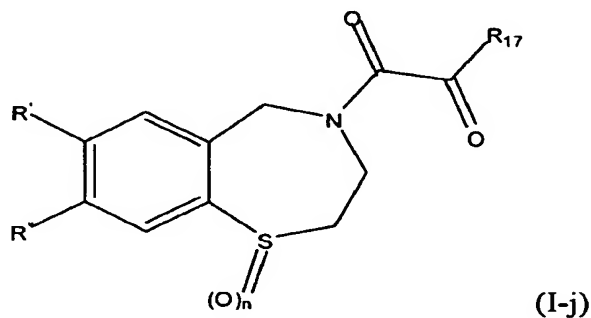
wherein R_{17} is selected from the group consisting of $-NR_{15}R_{16}$, $-NHN R_{15}R_{16}$, $-NHOH$, $-OR_{15}$, $-CH_2X$, alkenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each alkenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted;

each R , q , and n is as defined in compounds of formula I-a above; and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

[0046] In certain embodiments, the present invention provides compounds of formula I-i, wherein each R is independently selected from the group consisting of H, halogen, $-OH$, OMe , $-NH_2$, $-NO_2$, $-CN$, $-CF_3$, $-OCF_3$, $-N_3$, $-S(=O)_2C_1-C_4alkyl$, $-S(=O)C_1-C_4alkyl$, $-S-C_1-C_4alkyl$, $-OS(=O)_2CF_3$, Ph , $-NHCH_2Ph$, $-C(=O)Me$, $-OC(=O)Me$, morpholinyl and propenyl; and n is 0, 1, or 2.

[0047] In other embodiments, the present invention provides compounds of formula I-i, wherein R_{17} is $-NR_{15}R_{16}$, and $-OR_{15}$. In certain other embodiments, R_{17} is $-OH$, $-OMe$, $-NEt$, $-NHet$, $-NHPh$, $-NH_2$, or $-NHCH_2pyridyl$.

[0048] In one embodiment, the present invention provides compounds of formula of I-j:



wherein R' and R'' are independently selected from the group consisting of H, halogen, -OH, -NH₂, -NO₂, -CN, -CF₃, -OCF₃, -N₃, -SO₃H, -S(=O)₂alkyl, -S(=O)alkyl, -OS(=O)₂CF₃, acyl, alkyl, alkoxy, alkylamino, alkylthio, cycloalkyl, aryl, heterocyclyl, heterocyclalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino; and wherein each acyl, alkyl, alkoxy, alkylamino, cycloalkyl, aryl, heterocyclyl, heterocyclalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio may be substituted or unsubstituted;

R₁₇ is selected from the group consisting of -NR₁₅R₁₆, -NHNHNR₁₅R₁₆, -NHOH, -OR₁₅, -CH₂X, alkenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclalkyl; wherein each alkenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclalkyl may be substituted or unsubstituted;

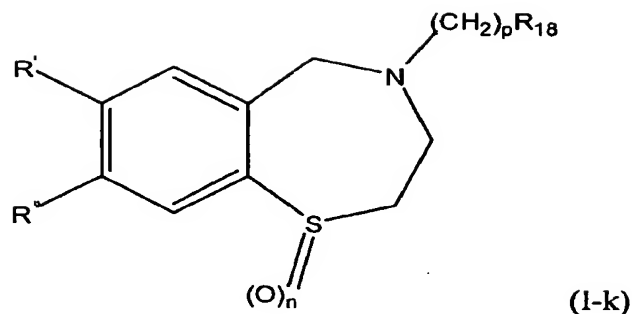
n is as defined in compounds of formula I-a; and

enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

[0049] In certain embodiments, the present invention provides compounds of formula I-j, wherein R' and R'' are independently selected from the group consisting of H, halogen, -OH, OMe, -NH₂, -NO₂, -CN, -CF₃, -OCF₃, -N₃, -S(=O)₂C₁-C₄alkyl, -S(=O)C₁-C₄alkyl, -S-C₁-C₄alkyl, -OS(=O)₂CF₃, Ph, -NHCH₂Ph, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1 or 3. In some cases, R' is H or OMe, and R'' is H.

[0050] In other embodiments, the present invention provides compounds of formula I-j, wherein R₁₇ is -NR₁₅R₁₆ or -OR₁₅. In certain other embodiments, R₁₇ is -OH, -OMe, -NEt, -NHEt, -NHPh, -NH₂, or -NHCH₂pyridyl.

[0051] In another embodiment, the methods of the present invention comprise administering to a subject a compound of Formula I-k, or enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, or prodrugs, or a combination thereof:



wherein R' and R'' are independently selected from the group consisting of H, halogen, -OH, -NH₂, -NO₂, -CN, -CF₃, -OCF₃, -N₃, -SO₃H, -S(=O)₂alkyl, -S(=O)alkyl, -OS(=O)₂CF₃, acyl, alkyl, alkoxyl, alkylamino, alkylthio, cycloalkyl, aryl, heterocyclyl, heterocyclalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylmino; and wherein each acyl, alkyl, alkoxyl, alkylamino, cycloalkyl, aryl, heterocyclyl, heterocyclalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio may be substituted or unsubstituted;

R₁₈ is selected from the group consisting of -NR₁₅R₁₆, -C(=O)NR₁₅R₁₆, -(C=O)OR₁₅, -OR₁₅, alkyl, aryl, cycloalkyl, heterocyclyl, and at one labeling group; wherein each alkyl, aryl, cycloalkyl, and heterocyclyl may be substituted or unsubstituted;

wherein p is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10;

and n is 0, 1, or 2;

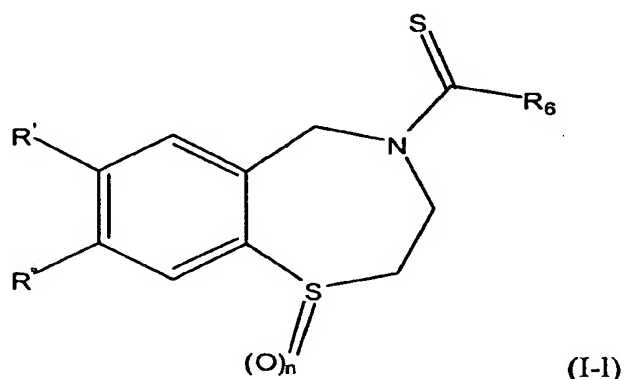
and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

[0052] In certain embodiments, the present invention provides compounds of formula I-k, wherein R' and R'' are independently selected from the group consisting of H, halogen, -OH, OMe, -NH₂, -NO₂, -CN, -CF₃, -OCF₃, -N₃, -S(=O)₂C₁-C₄alkyl, -S(=O)C₁-C₄alkyl, -S-C₁-C₄alkyl, -OS(=O)₂CF₃, Ph, -NHCH₂Ph, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1 or 3. In some cases, R' is H or OMe, and R'' is H.

[0053] In other embodiments, the present invention provides compounds of formula I-k, wherein R₁₈ is selected from the group consisting of -NR₁₅R₁₆, -(C=O)OR₁₅, -OR₁₅, alkyl, aryl, and at one labeling group; and wherein each alkyl and aryl may be substituted or unsubstituted. In some cases, m is 1, and R₁₈ is Ph, C(=O)OMe, C(=O)OH, aminoalkyl, NH₂, NHOH, or NHCbz. In other cases, m is 0, and R₁₈ is C₁-C₄ alkyl, such as Me, Et,

propyl, and butyl. In yet other cases, m is 2, and R₁₈ is pyrrolidine, piperidine, piperazine, or morpholine. In some embodiments, m is 3, 4, 5, 5, 7, or 8, and R₁₈ is a fluorescent labeling group selected from bodipy, dansyl, fluorescein, rhodamine, Texas red, cyanine dyes, pyrene, coumarins, Cascade Blue™, Pacific Blue, Marina Blue, Oregon Green, 4',6-Diamidino-2-phenylindole (DAPI), indopyra dyes, lucifer yellow, propidium iodide, porphyrins, arginine, and variants and derivatives thereof.

[0054] In yet another embodiment, the methods of the present invention comprise administering to a subject a compound of Formula I-I, or enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, or prodrugs, or a combination thereof:



wherein R' and R'' are independently selected from the group consisting of H, halogen, -OH, -NH₂, -NO₂, -CN, -CF₃, -OCF₃, -N₃, -SO₃H, -S(=O)₂alkyl, -S(=O)alkyl, -OS(=O)₂CF₃, acyl, alkyl, alkoxy, alkylamino, alkylthio, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylmino; and wherein each acyl, alkyl, alkoxy, alkylamino, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio may be substituted or unsubstituted;

R₆ and n are as defined in compounds of formula I-a;

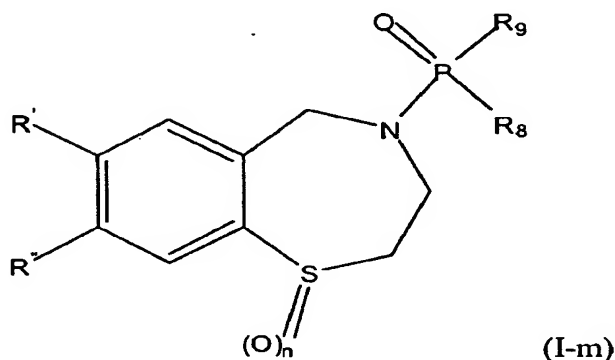
and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

[0055] In certain embodiments, the present invention provides compounds of formula I-I, wherein R' and R'' are independently selected from the group consisting of H, halogen, -OH, OMe, -NH₂, -NO₂, -CN, -CF₃, -OCF₃, -N₃, -S(=O)₂C₁-C₄alkyl, -S(=O)C₁-C₄alkyl, -S-

C₁-C₄alkyl, -OS(=O)₂CF₃, Ph, -NHCH₂Ph, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1 or 3. In some cases, R' is H or OMe, and R'' is H.

[0056] In other embodiments, the present invention provides compounds of formula I-l, wherein R₆ is selected from the group consisting of -NR₁₅R₁₆, -NHNHNR₁₅R₁₆, -OR₁₅, -NHOH, -CH₂X, acyl, alkenyl, alkyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkenyl, alkyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted. In some cases, R₆ is -NR₁₅R₁₆ such as -NHPh, pyrrolidine, piperidine, piperazine, morpholine, and the like. In some other cases, R₆ is alkoxyl, such as -O-tBu.

[0057] In a further embodiment, the methods of the present invention comprise administering to a subject a compound of Formula I-m, or enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, or prodrugs, or a combination thereof:



wherein R' and R'' are independently selected from the group consisting of H, halogen, -OH, -NH₂, -NO₂, -CN, -CF₃, -OCF₃, -N₃, -SO₃H, -S(=O)₂alkyl, -S(=O)alkyl, -OS(=O)₂CF₃, acyl, alkyl, alkoxyl, alkylamino, alkylthio, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino; and wherein each acyl, alkyl, alkoxyl, alkylamino, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio may be substituted or unsubstituted;

R₈, R₉ and n are as defined in compounds of formula I-a above; and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

[0058] In certain embodiments, the present invention provides compounds of formula **I-m**, wherein R' and R'' are independently selected from the group consisting of H, halogen, -OH, OMe, -NH₂, -NO₂, -CN, -CF₃, -OCF₃, -N₃, -S(=O)₂C₁-C₄alkyl, -S(=O)C₁-C₄alkyl, -S-C₁-C₄alkyl, -OS(=O)₂CF₃, Ph, -NHCH₂Ph, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1 or 3. In some cases, R' is H or OMe, and R'' is H.

[0059] In other embodiments, the present invention provides compounds of formula **I-m**, wherein R₈ and R₉ are independently alkyl, aryl, -OH, alkoxyl, or alkylamino. In some cases, R₈ is C₁-C₄ alkyl such as Me, Et, propyl and butyl; and R₉ is aryl such as phenyl.

[0060] In one embodiment, the compound is selected from S1, S2, S3, S4, S5, S6, S7, S9, S11, S12, S13, S14, S19, S20, S22, S23, S25, S26, S36, S37, S38, S40, S43, S44, S45, S46, S47, S48, S49, S50, S51, S52, S53, S54, S55, S56, S57, S58, S59, S60, S61, S62, S63, S64, S66, S67, S68, S69, S70, S71, S72, S73, S74, S75, S76, S77, S78, S79, S80, S81, S82, S83, S84, S85, S86, S87, S88, S89, S90, S91, S92, S93, S94, S95, S96, S97, S98, S99, S100, S101, S102, S103, S104, S105, S107, S108, S109, S110, S111, S112, S113, S114, S115, S116, S117, S118, S119, S120, S121, S122, or S123, or enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, or prodrugs, or a combination thereof.

[0061] In one embodiment of the methods for treating a non-human animal subject, the method comprises administering to the animal subject an agent, wherein the agent is S36 or salts, hydrates, solvates, complexes or prodrugs thereof. In another embodiment, the agent is S64 or salts, hydrates, solvates, complexes or prodrugs thereof. In yet other embodiments, the agent is selected from the group consisting of: S47, S50, S64, S74, S75, S77, S101, S102 and S103 and salts, hydrates, solvates, complexes and prodrugs thereof.

[0062] The compounds of the invention may optionally comprise a labeling group, such as a fluorescent, bioluminescent, chemiluminescent, colorimetric or radioactive labeling group. Suitable fluorescent labeling groups include, but are not limited to, bodipy, dansyl, fluorescein, rhodamine, Texas red, cyanine dyes, pyrene, coumarins, Cascade BlueTM, Pacific Blue, Marina Blue, Oregon Green, 4', 6-Diamidino-2-phenylindole (DAPI), indopyra dyes, lucifer yellow, propidium iodide, porphyrins, and variants and derivatives thereof. One of skill in the art can readily select a suitable marker or labeling group, and

conjugate such a labeling group to any of the compounds of the invention, without undue experimentation.

[0063] In certain embodiments of the method for treating a disorder or disease in a non-human animal, the disease or disorder treated by the methods of the invention can be an arrhythmogenic condition, wherein the arrhythmogenic condition can be selected but is not limited to conditions from the group consisting of: sudden cardiac death, arrhythmogenic right ventricular cardiomyopathy, hypertrophic cardiomyopathy, dilated cardiomyopathy, restrictive cardiomyopathy, ventricular arrhythmias, sick sinus syndrome, atrial standstill, atrial fibrillation, sinus tachycardia, and ventricular fibrillations. In certain embodiments, non-human animal is selected from the group consisting of canines, felines, equines, porcine, poultry, ruminants, and rodents.

[0064] In certain embodiments of the method for treating a disorder or disease in a non-human animal, the agent mimics binding of the calstabin protein to the RyR receptor. In certain other embodiment, the agent reduces or prevents PKA-phosphorylation of the RyR receptor. In certain other embodiments, the agent prevents or inhibits dissociation of the calstabin protein from the RyR receptor. In certain other embodiments, the agent increases binding of the calstabin protein to the RyR receptor. In certain other embodiments, the agent increases the ratio of calstabin protein to RyR receptor in the RyR receptor-calstabin complex.

[0065] In certain embodiments of the method for treating a disorder or disease in a non-human animal, the disease or disorder is such that the non-human animal demonstrates a reduced level of calstabin2 mRNA in the cardiac tissue compared to a level of calstabin2 mRNA in cardiac tissue of a control non-human animal known not to have arrhythmogenic right ventricular cardiomyopathy. In other embodiments of the method for treating a disorder or disease in a non-human animal, the disease or disorder is such that the non-human animal demonstrates a reduced level of calstabin2 protein in the RyR2-calstabin2 protein complex in cardiac tissue of a subject non-human animal compared to a level of calstabin2 protein in the RyR2-calstabin2 protein complex in cardiac tissue of a control non-human animal known not to have arrhythmogenic right ventricular cardiomyopathy. In certain embodiments, the non-human animal can be selected from, but is not limited to, the group consisting of canines, felines, equines, porcine, poultry, ruminants, and rodents.

[0066] In certain embodiments, the methods of the present invention are directed to methods of diagnosis, and/or treatment of diseases or disorders, wherein the RyR receptor is an RyR2 receptor, and the calstabin protein is a calstabin 2 (FKBP12.6) protein. In other embodiments, the RyR receptor is an RyR1 receptor, and the calstabin protein is a calstabin1 (FKBP12) protein. In other embodiments, the RyR receptor is an RyR3 receptor and the calstabin protein is a calstabin1 (FKBP12) protein.

[0067] In another aspect, the invention provides a method for treating or preventing Sudden Cardiac Death in a non-human animal, the method comprising administering to the non-human animal a veterinary effective amount of an agent known to affect the interaction of a calstabin2 (FKBP12.6) protein to an RyR2 receptor in the RyR2-calstabin2 protein complex in cardiac tissue of the non-human animal. In certain embodiments, the non-human animal can be selected from, but is not limited to, the group consisting of canines, felines, equines, porcine, poultry, ruminants, and rodents.

[0068] In another aspect, the invention provides a method for treating or preventing arrhythmogenic right ventricular cardiomyopathy (ARVC) in a non-human animal, including but not limited to a canine, comprising administering to the non-human animal a pharmaceutically effective amount of an agent known to regulate binding of a calstabin2 (FKBP12.6) protein to an RyR2 receptor in cardiac tissue of the non-human animal. In non-limiting example, the canine is selected from a breed of Boxer, German Shepherd, Miniature Schnauzer, West Highland White terrier, Dachshund, English Springer Spaniel, Golden Retriever, Doberman Pinscher, Newfoundland, Cocker Spaniel, Grate Dane, Irish Wolfhound, Afghan Hound, and Saluki.

[0069] In certain embodiments of the methods for treating or preventing a disease or disorder, including but not limited to Sudden Cardiac Death in a non-human animal, and the methods for treating or preventing arrhythmogenic right ventricular cardiomyopathy (ARVC) in a non-human animal, such as a canine, the agent is selected from the group of compounds of the general formula I, I-a, I-b, I-c, I-d, I-e, I-f, I-g, I-h, I-i, I-j, I-k, I-l and I-m, or enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, or prodrugs, or a combination thereof. In one embodiment, the compound is selected from S1, S2, S3, S4, S5, S6, S7, S9, S11, S12, S13, S14, S19, S20, S22, S23, S25, S26, S36, S37, S38, S40, S43, S44, S45, S46, S47, S48, S49, S50, S51, S52, S53, S54, S55, S56, S57, S58, S59, S60, S61, S62, S63, S64, S66, S67, S68, S69, S70, S71,

S72, S73, S74, S75, S76, S77, S78, S79, S80, S81, S82, S83, S84, S85, S86, S87, S88, S89, S90, S91, S92, S93, S94, S95, S96, S97, S98, S99, S100, S101, S102, S103, S104, S105, S107, S108, S109, S110, S111, S112, S113, S114, S115, S116, S117, S118, S119, S120, S121, S122, or S123, or enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, or prodrugs, or a combination thereof.

[0070] In non-limiting examples, the agent of the methods for treating or preventing arrhythmogenic conditions in a non-human animal, and arrhythmogenic right ventricular cardiomyopathy (ARVC) in a canine, is an agent selected from the group of compounds of the general formula I, I-a, I-b, I-c, I-d, I-e, I-f, I-g, I-h, I-i, I-j, I-k, I-l and I-m, or enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, or prodrugs, or a combination thereof. In one embodiment, the compound is selected from S1, S2, S3, S4, S5, S6, S7, S9, S11, S12, S13, S14, S19, S20, S22, S23, S25, S26, S36, S37, S38, S40, S43, S44, S45, S46, S47, S48, S49, S50, S51, S52, S53, S54, S55, S56, S57, S58, S59, S60, S61, S62, S63, S64, S66, S67, S68, S69, S70, S71, S72, S73, S74, S75, S76, S77, S78, S79, S80, S81, S82, S83, S84, S85, S86, S87, S88, S89, S90, S91, S92, S93, S94, S95, S96, S97, S98, S99, S100, S101, S102, S103, S104, S105, S107, S108, S109, S110, S111, S112, S113, S114, S115, S116, S117, S118, S119, S120, S121, S122, or S123, or enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, or prodrugs, or a combination thereof.

[0071] In certain embodiments of the methods for treating or preventing arrhythmogenic conditions in a non-human animal, administering the agent prevents or reduces the occurrence or severity of ventricular arrhythmias. In other embodiments, administering the agent prevents or reduces the occurrence of sudden cardiac death.

[0072] In certain embodiments of the methods for treating or preventing Sudden Cardiac Death or arrhythmogenic right ventricular cardiomyopathy (ARVC) in a non-human animal, which includes but is not limited to a canine, the non-human animal demonstrates a reduced level of calstabin2 mRNA in the cardiac tissue compared to a level of calstabin2 mRNA in cardiac tissue of a control non-human animal known not to have arrhythmogenic right ventricular cardiomyopathy. In other embodiments, the non-human animal demonstrates a reduced level of calstabin2 in an RyR2-calstabin2 complex in the cardiac tissue compared to a level of calstabin2 in an RyR2-calstabin2 complex in cardiac tissue of a control non-human animal known not to have arrhythmogenic right ventricular cardiomyopathy.

[0073] In another aspect, the invention provides a method for determining the effect of a test compound on an arrhythmogenic condition in a subject, wherein the arrhythmogenic condition is selected from the group consisting of: sudden cardiac death, arrhythmogenic right ventricular cardiomyopathy, hypertrophic cardiomyopathy, dilated cardiomyopathy, restrictive cardiomyopathy, ventricular arrhythmias, sick sinus syndrome, atrial standstill, atrial fibrillation, sinus tachycardia, and ventricular fibrillations. In certain embodiments, the method of determining the effect of a test compound on an arrhythmogenic condition in a subject comprises the steps of: (1) administering a placebo compound to a non-human animal, wherein the animal is characterized by decreased calstabin2 levels in cardiac tissue, and (2) administering a test compound to a second non-human animal, wherein the second animal is characterized by decreased calstabin2 levels in cardiac tissue, and (3) determining the rate of electrical signals that control the heartbeat rhythm in the presence of the placebo and the test compound, wherein a test compound that improves irregular heartbeat rhythm in the second animal compared with the first animal, is indicative of a test compound that prevents or treats cardiac arrhythmias, and wherein the non-human animal is not a mouse.

[0074] The step of determining the rate of electrical signals that control the heartbeat rhythm can be done any suitable method known in the art, including but not limited to measurement by electrocardiogram. In certain embodiments of the method for determining the effect of a test compound on an arrhythmogenic condition, the subject is a non-human animal can be selected from but is not limited to the group of: canines, felines, equines, porcine, poultry, ruminants, and rodents.

[0075] In other aspects, the invention is directed to a synthetic nucleic acid comprising from about 10 to about 30 consecutive nucleotides from SEQ ID NO: 1. In another aspect, the invention is directed to a synthetic nucleic acid comprising from about 10 to about 30 consecutive nucleotides from a sequence which is complementary to SEQ ID NO: 1. In certain embodiments, the invention is directed to a composition, comprising one or more of the synthetic nucleic acid sequences which comprise from about 10 to about 30 consecutive nucleotides from SEQ ID NO: 1, or from a sequence which is complementary to SEQ ID NO: 1. In one embodiment, the composition of nucleic acids comprises at least one nucleic acid which has from about 10 to about 30 consecutive nucleotides derived each from SEQ ID NO:1, and a sequence which is complementary to SEQ ID NO: 1.

[0076] In another aspect, the invention is directed to primer set comprising at least two synthetic nucleic acid sequences, wherein at least one of the at least two synthetic nucleic acid sequences is selected from synthetic nucleic acid comprising from about 10 to about 30 consecutive nucleotides from SEQ ID NO: 1, or from about 10 to about 30 consecutive nucleotides from a sequence which is complementary to SEQ ID NO: 1.

[0077] In another aspect, the invention is directed to a kit for determining the levels of calstabin2 mRNA in a sample, comprising at least one of the synthetic nucleic acid sequences of the invention. The kit of the invention can further comprise optional PCR reagents and positive/negative control for determining the levels of calstabin2 mRNA in a sample derived from tissue of a non-human animal.

[0078] In another aspect, the invention is directed to a method for determining whether a test compound affects the interaction between calstabin2 and RyR2 in cardiac myocyte cells characterized by reduced levels of calstabin2, the method comprising: (1) contacting cardiac myocyte cell which has reduced levels of calstabin2 with a test compound, (2) determining the levels of calstabin2 in the RyR2-calstabin complex in cardiac myocyte cell, wherein increased level of calstabin2 in the RyR2-calstabin2 complex, after the cardiac myocyte cell has been contacted with a test agent, compared to the calstabin2 levels in the RyR2-calstabin complex in a cardiac myocyte cell which has not been contacted with the test compound, is indicative of an agent which affects the interaction between calstabin2 and RyR2. In another embodiment, the effect of a test compound in the interaction between calstabin2 and RyR2 can be determined by measuring the levels of release of intracellular stores of Ca^{2+} , wherein a test compound which repairs Ca^{2+} leak, is indicative of a compound which affects the interaction between calstabin2 and RyR2.

[0079] In another aspect, the invention is directed to an animal feed comprising an agent selected from the group of compounds of the general formula I, I-a, I-b, I-c, I-d, I-e, I-f, I-g, I-h, I-i, I-j, I-k, I-l and I-m, or enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, or prodrugs, or a combination thereof. In one embodiment, the compound is selected from S1, S2, S3, S4, S5, S6, S7, S9, S11, S12, S13, S14, S19, S20, S22, S23, S25, S26, S36, S37, S38, S40, S43, S44, S45, S46, S47, S48, S49, S50, S51, S52, S53, S54, S55, S56, S57, S58, S59, S60, S61, S62, S63, S64, S66, S67, S68, S69, S70, S71, S72, S73, S74, S75, S76, S77, S78, S79, S80, S81, S82, S83, S84, S85, S86, S87, S88, S89, S90, S91, S92, S93, S94, S95, S96, S97, S98, S99, S100, S101, S102,

S103, S104, S105, S107, S108, S109, S110, S111, S112, S113, S114, S115, S116, S117, S118, S119, S120, S121, S122, or S123, or enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, or prodrugs, or a combination thereof.

[0080] In certain embodiments, the animal feed is useful in methods for treatment of arrhythmogenic condition in a non-human animal. In other embodiments, the feed is useful for the treatment or prevention of diseases and disorders associated with reduced levels of calstabin in an RyR-calstabin protein complex. The animal feed is in a form that is palatable to the non-human animal that is the subject of the treatment and prevention methods of the invention. Non-limiting examples of the inventive animal feed are biscuits, treats, nutritional pellets, which are provided in a formulation that is suitable for the subject non-human animal.

BRIEF DESCRIPTION OF THE FIGURES

[0081] Figure 1 shows that affected Boxer dogs have decreased calstabin2 in a cardiac tissue and in the RyR2 channel complex. Cardiac lysates were prepared from control animals and affected boxer dogs as described in the Examples. (A) Cardiac lysates (200 μ g) were separated by 15% PAGE. Immunoblots were developed with anti-calstabin antibody and show the levels of calstabin 1 and 2 in the cardiac lysates. (B) RyR2 was immunoprecipitated from both control and boxer (Cardiomyopathy) cardiac lysates (250 μ g). The immunoprecipitates were separated by either 6% (RyR2) or 15% (Calstabin) PAGE. Depicted are immunoblots of total RyR2, PKA phosphorylated RyR2, and Calstabin 2 in the complex.

[0082] Figure 2A shows SEQ ID NO.: 1, which is cDNA sequence of canine FKBP12.6, and Figure 2B shows SEQ ID NO.: 2, which is amino sequence of canine FKBP12.6.

[0083] Figure 3, embodiments A, B, C, and D are, respectively, (A) immunoblots of PKA phosphorylated RyR2 in the presence of FKBP12.6 and increasing JTV-519 concentrations; (B) immunoblots of PKA phosphorylated RyR2 in the presence of 0.5 nM S36; (C) a graph of current through plasma membrane, voltage dependent L-type Ca^{2+} channels which are completely blocked by nifedipine but not by S36 in isolated mouse cardiomyocytes; and (D) a graph of the voltage-dependence of L-type Ca^{2+} current in channels in the presence of JTV-519 and S36.

[0084] Figure 4, embodiments A, B, C, and D demonstrate the prevention of exercise-induced ventricular arrhythmias by JTV-519 in haploinsufficient calstabin2 (FKBP12.6)^{+/-} mice. Embodiment A are representative telemetric electrocardiograms (ECGs) of an untreated calstabin2 (FKBP12.6)^{+/-} mouse (left), a JTV-519-treated calstabin2 (FKBP12.6)^{+/-} mouse (middle), and a calstabin2 (FKBP12.6)^{-/-} mouse (right). Embodiment B are telemetry recordings of a sustained polymorphic ventricular tachycardia (sVT) in (upper) an untreated haploinsufficient calstabin2 (FKBP12.6)^{+/-} mouse and (lower) a JTV-519-treated calstabin2 (FKBP12.6)^{+/-} mouse, each subjected to exercise testing immediately followed by injection with 0.5 mg epinephrine per kilogram of body weight. Embodiment C are graphs showing the numbers of mice with cardiac death (left), sustained VTs (middle), and nonsustained VTs (right) in experimental groups of mice subjected to exercise testing and injection with 0.5 mg/kg epinephrine. Embodiment D are graphs comparing the dose dependence of pharmacological effects of JTV-519 and S36 in regard to sudden cardiac death (left), sustained VTs (middle), and nonsustained VTs (right).

[0085] Figure 5 is a graph showing fractional shortening (FS) of the left ventricle assessed by M-mode echocardiography 2 weeks post-myocardial infarction in placebo versus S36-treated mice. S36 treated mice show a significant improvement in FS in the 100 nM and 200 nM groups as compared to placebo.

[0086] Figure 6 is a graph showing heart weight to body weight (HW/BW) ratios and pressure-volume loops quantifications (dP/dt) one week post-myocardial infarction of placebo and S36-treated mice. S36 treatment results in a beneficial reduction of the HW/BW ratio and increased velocity of pressure development in S36 as compared to placebo treated mice.

[0087] Figure 7 is a graph summarizing EC₅₀ values of JTV-519 and a series of Rycal compounds indicating several compounds with a higher biologic activity as evidenced by significantly lower EC₅₀ values compared to JTV-519.

[0088] Figure 8 shows representative lead II ECG from a Boxer dog with ARVC showing non-sustained ventricular tachycardia. 25mm/sec, 0.5cm/mV. This demonstrates that affected Boxer dogs manifest ventricular arrhythmias with left-bundle branch block morphology.

[0089] Figure 9 shows representative histopathological examination of right ventricular tissue from a dog with ARVC that reveals substantial cardiac myocyte loss and replacement with adipose tissue. The sample is positioned with the epicardial surface of the right ventricle at the top of the photomicrograph. 20X magnification, hemotoxylin and eosin. This demonstrates that affected Boxer dogs display histological abnormalities.

DETAILED DESCRIPTION

[0090] As used herein the term “arrhythmogenic condition” refers to a cardiac condition which includes but is not limited to heart failure, sudden cardiac death, cardiac arrhythmias, e.g., ventricular and atrial tachycardia; atrial arrhythmia, including atrial tachyarrhythmia and atrial fibrillation (both sustained and non-sustained); ventricular arrhythmia, including ventricular tachyarrhythmia, ventricular fibrillation; and stress or exercise-induced cardiac arrhythmia, catecholaminergic polymorphic ventricular tachycardia (CPVT), arrhythmogenic right ventricular cardiomyopathy (ARVD/C), hyperthrophic cardiomyopathy, dilated cardiomyopathy, restrictive cardiomyopathy, sick sinus syndrome, atrial standstill sinus tachycardia, and/or stress- or exercise-induced sudden cardiac death.

[0091] As used herein, the term “arrhythmogenic right ventricular cardiomyopathy” (ARVC) encompasses arrhythmogenic right ventricular dysplasia (ARVD) and several other clinical entities, some of them known by a different name. The terms ARVD/C and ARVC are used interchangeably.

[0092] A "candidate" for a cardiac condition (e.g., cardiac arrhythmia or heart failure) is a subject, human or non-human animal, who is known to be, or is believed to be, or is suspected of being, at risk for developing a cardiac condition. Examples of candidates for a cardiac condition include, without limitation, a non-human animal or person suspected of having cardiac arrhythmia (e.g., tachycardia; atrial arrhythmia, including atrial tachyarrhythmia and atrial fibrillation (both sustained and non-sustained); ventricular arrhythmia, including ventricular fibrillation; and exercise-induced cardiac arrhythmia) and/or heart failure; and an animal/person who is known to be, or is believed to be, or is suspected of being, at risk for developing cardiac arrhythmia (e.g., tachycardia; atrial arrhythmia, including atrial tachyarrhythmia and atrial fibrillation (both sustained and non-sustained); ventricular arrhythmia, including ventricular fibrillation; and exercise-induced cardiac arrhythmia) and/or heart failure.

[0093] A "candidate" for stress or exercise-induced cardiac arrhythmia is a subject, human or non-human animal, who is known to be, or is believed to be, or is suspected of being, at risk for developing cardiac arrhythmia during/after physical exercise. Examples of candidates for exercise-induced cardiac arrhythmia include, without limitation, a non-human animal or a person known to have catecholaminergic polymorphic ventricular tachycardia (CPVT), or arrhythmogenic right ventricular cardiomyopathy (ARVD/C); a person or non-human animal suspected of having CPVT, or arrhythmogenic right ventricular cardiomyopathy (ARVD/C); and a person or non-human animal who is known to be, or is believed to be, or is suspected of being, at risk for developing cardiac arrhythmia during/after physical exercise, and who is about to exercise, is currently exercising, or has just completed exercise. As discussed herein, arrhythmogenic right ventricular cardiomyopathy (ARVD/C), can be characterized by progressive fibro-fatty displacement of the myocardium in the right ventricle. ARVD/C subjects may suffer arrhythmias, heart failure and sudden death due to the functional exclusion of the right ventricle. CPVT is an inherited disorder in individuals with structurally-normal hearts. It is characterized by stress-induced ventricular tachycardia--a lethal arrhythmia that may cause sudden cardiac death. In subjects with CPVT, physical exertion and/or stress induce bidirectional and/or polymorphic ventricular tachycardias that lead to sudden cardiac death (SCD) in the absence of detectable structural heart disease. Individuals with CPVT have ventricular arrhythmias when subjected to exercise, but do not develop arrhythmias at rest.

[0094] As used herein and in the appended claims, the singular forms "a," "an," and "the" include plural references unless the content clearly dictates otherwise. Thus, for example, reference to "an agent" includes a plurality of such agents and equivalents thereof known to those skilled in the art, and reference to "the FKBP12.6 polypeptide" is a reference to one or more FKBP12.6 polypeptides (also known as calstabin2) and equivalents thereof known to those skilled in the art, and so forth. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety.

[0095] The following are definitions of terms used in the present specification. The initial definition provided for a group or term herein applies to that group or term throughout the present specification individually or as part of another group, unless otherwise indicated.

[0096] As used herein, the term "RyCal compounds" refers to compounds of the general Formula I, I-a, I-b, I-c, I-d, I-e, I-f, I-g, I-h, I-i, I-j, I-k, I-l, I-m or II as provided by the invention, and herein referred to as "compound(s) of the invention".

[0097] The compounds of the invention are referred using a numerical naming system, with compound numbers 1 to 123 provided herein. These numbered compounds are referred to using either the prefix "S" or the prefix "ARM." Thus, the first numbered compound is referred to either as "S1" or "ARM001", the second numbered compound is referred to as either "S2" or "ARM002", the third numbered compound is referred to as either "S3" or "ARM003", and so on. The "S" and the "ARM" nomenclature systems are used interchangeably throughout the specification, the drawings, and the claims.

[0098] The terms "salt" and "pharmaceutically acceptable salt", as used herein, refer to salts that are suitable for, or compatible with, the treatment of subjects such as non-human animals. The salts can be any non-toxic organic or inorganic salt of any of the compounds represented by Formula I, I-a, I-b, I-c, I-d, I-e, I-f, I-g, I-h, I-i, I-j, I-k, I-l or I-m, or any of the specific compounds described herein, or any of their intermediates. Illustrative salt-forming ions include, but are not limited to, ammonium (NH_4^+), calcium (Ca^{2+}), iron (Fe^{2+} and Fe^{3+}), magnesium (Mg^{2+}), potassium (K^+), pyridinium ($\text{C}_5\text{H}_5\text{NH}^+$), quaternary ammonium (NR_4^+), sodium (Na^+), acetate, carbonate, chloride, bromide, citrate, cyanide, hydroxide, nitrate, nitrite, oxide, phosphate, sulfate, maleate, fumarate, lactate, tartrate, gluconate, besylate, and valproate. Illustrative inorganic acids that form suitable salts include, but are not limited to, hydrochloric, hydrobromic, sulfuric and phosphoric acids, as well as metal salts such as sodium monohydrogen orthophosphate and potassium hydrogen sulfate. Illustrative organic acids that form suitable acid addition salts include, but are not limited to, mono-, di-, and tricarboxylic acids such as glycolic, lactic, pyruvic, malonic, succinic, glutaric, fumaric, malic, tartaric, citric, ascorbic, maleic, benzoic, phenylacetic, cinnamic and salicylic acids, as well as sulfonic acids such as p-toluene sulfonic and methanesulfonic acids. Either mono or di-acid salts can be formed, and such salts exist in either a hydrated, solvated or substantially anhydrous form. In general, the acid addition salts of compounds of Formula I, I-a, I-b, I-c, I-d, I-e, I-f, I-g, I-h, I-i, I-j, I-k, I-l and I-m, are more soluble in water and various hydrophilic organic solvents, and generally demonstrate higher melting points in comparison to their free base forms. The selection of an appropriate salt can be performed by one skilled in the art. For example, one can select

salts in reference to "Handbook of Pharmaceutical Salts : Properties, Selection, and Use" by P. Heinrich Stahl and Camille G. Wermuth, or Berge (1977) "Pharmaceutcial Salts" J. Pharm Sci., Vol 66(1), p 1-19. Other non-pharmaceutically acceptable salts (*e.g.*, oxalates) may be used, for example, in the isolation of compounds of the invention for laboratory use or for subsequent conversion to a pharmaceutically acceptable acid addition salt.

[0099] The term "alkyl" as used herein refers to a linear or branched, saturated hydrocarbon having from 1 to 6 carbon atoms. Representative alkyl groups include, but are not limited to, methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, tert-butyl, pentyl, isopentyl, neopentyl, hexyl, isohexyl, and neohexyl. The term "C₁-C₄ alkyl" refers to a straight or branched chain alkane (hydrocarbon) radical containing from 1 to 4 carbon atoms, such as methyl, ethyl, propyl, isopropyl, n-butyl, t-butyl, and isobutyl.

[00100] The term "alkenyl" as used herein refers to a linear or branched hydrocarbon having from 2 to 6 carbon atoms and having at least one carbon-carbon double bond. In one embodiment, the alkenyl has one or two double bonds. The alkenyl moiety may exist in the E or Z conformation and the compounds of the present invention include both conformations.

[00101] The term "alkynyl" as used herein refers to a linear or branched hydrocarbon having from 2 to 6 carbon atoms and having at least one carbon-carbon triple bond.

[00102] The term "aryl" as used herein refers to an aromatic group containing 1 to 3 aromatic rings, either fused or linked.

[00103] The term "cyclic group" as used herein includes a cycloalkyl group and a heterocyclic group.

[00104] The term "cycloalkyl group" as used herein refers to a three- to seven-membered saturated or partially unsaturated carbon ring. Any suitable ring position of the cycloalkyl group may be covalently linked to the defined chemical structure. Exemplary cycloalkyl groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and cycloheptyl.

[00105] The term "halogen" as used herein refers to fluorine, chlorine, bromine, and iodine.

[00106] The term "heterocyclic group" or "heterocyclic" or "heterocyclyl" or "heterocyclo" as used herein refers to fully saturated, or partially or fully unsaturated, including aromatic (i.e., "heteroaryl") cyclic groups (for example, 4 to 7 membered monocyclic, 7 to 11 membered bicyclic, or 10 to 16 membered tricyclic ring systems) which have at least one heteroatom in at least one carbon atom-containing ring. Each ring of the heterocyclic group containing a heteroatom may have 1, 2, 3, or 4 heteroatoms selected from nitrogen atoms, oxygen atoms and/or sulfur atoms, where the nitrogen and sulfur heteroatoms may optionally be oxidized and the nitrogen heteroatoms may optionally be quaternized. The heterocyclic group may be attached to the remainder of the molecule at any heteroatom or carbon atom of the ring or ring system. Exemplary heterocyclic groups include, but are not limited to, azepanyl, azetidiny, aziridiny, dioxolanyl, furanyl, furazanyl, homo piperaziny, imidazolidiny, imidazoliny, isothiazolyl, isoxazolyl, morpholiny, oxadiazolyl, oxazolidiny, oxazolyl, oxazolidiny, pyrimidiny, phenanthridiny, phenanthroliny, piperaziny, piperidiny, pyranyl, pyraziny, pyrazolidiny, pyrazoliny, pyrazolyl, pyridaziny, pyridooxazolyl, pyridoimidazolyl, pyridothiazolyl, pyridiny, pyrimidiny, pyrrolidiny, pyrroliny, quinuclidiny, tetrahydrofuranyl, thiadiaziny, thiadiazolyl, thienyl, thienothiazolyl, thienooxazolyl, thienoimidazolyl, thiomorpholiny, thiophenyl, triaziny, and triazolyl. Exemplary bicyclic heterocyclic groups include indolyl, isoindolyl, benzothiazolyl, benzoxazolyl, benzoxadiazolyl, benzothieryl, quinuclidiny, quinoliny, tetrahydroisoquinoliny, isoquinoliny, benzimidazolyl, benzopyranyl, indoliziny, benzofuryl, benzofurazanyl, chromonyl, coumariny, benzopyranyl, cinnoliny, quinoxaliny, indazolyl, pyrrolopyridyl, furopyridiny (such as furo[2,3-c]pyridiny, furo[3,2-b]pyridiny] or furo[2,3-b]pyridiny), dihydroisoindolyl, dihydroquinazoliny (such as 3,4-dihydro-4-oxo-quinazoliny), triazinylazepiny, tetrahydroquinoliny and the like. Exemplary tricyclic heterocyclic groups include carbazolyl, benzidolyl, phenanthroliny, acridiny, phenanthridiny, xanthenyl and the like.

[00107] The term "phenyl" as used herein refers to a substituted or unsubstituted phenyl group.

[00108] The aforementioned terms "alkyl," "alkenyl," "alkynyl," "aryl," "phenyl," "cyclic group," "cycloalkyl," "heterocyclyl," "heterocyclo," and "heterocycle" may further be optionally substituted with one or more substituents. Exemplary substituents include but

are not limited to one or more of the following groups: hydrogen, halogen, CF_3 , OCF_3 , cyano, nitro, N_3 , oxo, cycloalkyl, alkenyl, alkynyl, heterocycle, aryl, alkylaryl, heteroaryl, OR_a , SR_a , S(=O)R_c , $\text{S(=O)}_2\text{R}_c$, $\text{P(=O)}_2\text{R}_c$, $\text{S(=O)}_2\text{OR}_a$, $\text{P(=O)}_2\text{OR}_a$, NR_bR_c , $\text{NR}_b\text{S(=O)}_2\text{R}_c$, $\text{NR}_b\text{P(=O)}_2\text{R}_c$, $\text{S(=O)}_2\text{NR}_b\text{R}_c$, $\text{P(=O)}_2\text{NR}_b\text{R}_c$, C(=O)OR_a , C(=O)R_a , $\text{C(=O)NR}_b\text{R}_c$, OC(=O)R_a , $\text{OC(=O)NR}_b\text{R}_c$, $\text{NR}_b\text{C(=O)OR}_a$, $\text{NR}_d\text{C(=O)NR}_b\text{R}_c$, $\text{NR}_d\text{S(=O)}_2\text{NR}_b\text{R}_c$, $\text{NR}_d\text{P(=O)}_2\text{NR}_b\text{R}_c$, $\text{NR}_b\text{C(=O)R}_a$, or $\text{NR}_b\text{P(=O)}_2\text{R}_c$, wherein R_a is hydrogen, alkyl, cycloalkyl, alkenyl, alkynyl, alkylaryl, heteroaryl, heterocycle, or aryl; R_b , R_c and R_d are independently hydrogen, alkyl, cycloalkyl, alkylaryl, heteroaryl, heterocycle, aryl, or said R_b and R_c together with the N to which they are bonded optionally form a heterocycle; and R_e is alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, alkylaryl, heteroaryl, heterocycle, or aryl. In the aforementioned exemplary substituents, groups such as alkyl, cycloalkyl, alkenyl, alkynyl, cycloalkenyl, alkylaryl, heteroaryl, heterocycle and aryl can themselves be optionally substituted.

[00109] Exemplary substituents may further optionally include at least one labeling group, such as a fluorescent, a bioluminescent, a chemiluminescent, a colorimetric and a radioactive labeling group. A fluorescent labeling group can be selected from bodipy, dansyl, fluorescein, rhodamine, Texas red, cyanine dyes, pyrene, coumarins, Cascade BlueTM, Pacific Blue, Marina Blue, Oregon Green, 4',6-Diamidino-2-phenylindole (DAPI), indopyra dyes, lucifer yellow, propidium iodide, porphyrins, arginine, and variants and derivatives thereof. For example, ARM118 of the present invention contains a labeling group BODIPY, which is a family of fluorophores based on the 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene moiety. For further information on fluorescent label moieties and fluorescence techniques, see, e.g., *Handbook of Fluorescent Probes and Research Chemicals*, by Richard P. Haugland, Sixth Edition, Molecular Probes, (1996), which is hereby incorporated by reference in its entirety. One of skill in the art can readily select a suitable labeling group, and conjugate such a labeling group to any of the compounds of the invention, without undue experimentation.

[00110] The term "quaternary nitrogen" refers to a tetravalent positively charged nitrogen atom including, for example, the positively charged nitrogen in a tetraalkylammonium group (e.g., tetramethylammonium, N-methylpyridinium), the positively charged nitrogen in protonated ammonium species (e.g., trimethylhydroammonium, N-hydropyridinium), the positively charged nitrogen in amine N-oxides

(e.g., N-methyl-morpholine-N-oxide, pyridine-N-oxide), and the positively charged nitrogen in an N-amino-ammonium group (e.g., N-aminopyridinium).

[00111] Throughout the specification, unless otherwise noted, the nitrogen in the benzothiazepine ring of compounds of the present invention may optionally be a quaternary nitrogen. Non-limiting examples include ARM-113 and ARM-119.

[00112] Compounds of the present invention may exist in their tautomeric form (for example, as an amide or imino ether). All such tautomeric forms are contemplated herein as part of the present invention.

[00113] The term "prodrug" as employed herein denotes a compound that, upon administration to a subject, undergoes chemical conversion by metabolic or chemical processes to yield compounds of the present invention.

[00114] All stereoisomers of the compounds of the present invention (for example, those which may exist due to asymmetric carbons on various substituents), including enantiomeric forms and diastereomeric forms, are contemplated within the scope of this invention. Individual stereoisomers of the compounds of the invention may, for example, be substantially free of other isomers (e.g., as a pure or substantially pure optical isomer having a specified activity), or may be admixed, for example, as racemates or with all other, or other selected, stereoisomers. The chiral centers of the present invention may have the S or R configuration as defined by the IUPAC 1974 Recommendations. The racemic forms can be resolved by physical methods, such as, for example, fractional crystallization, separation or crystallization of diastereomeric derivatives or separation by chiral column chromatography. The individual optical isomers can be obtained from the racemates by any suitable method, including without limitation, conventional methods, such as, for example, salt formation with an optically active acid followed by crystallization.

[00115] Compounds of the present invention are, subsequent to their preparation, preferably isolated and purified to obtain a composition containing an amount by weight equal to or greater than 99% of the compound ("substantially pure" compound), which is then used or formulated as described herein. Such "substantially pure" compounds of the present invention are also contemplated herein as part of the present invention.

[00116] All configurational isomers of the compounds of the present invention are contemplated, either in admixture or in pure or substantially pure form. The definition of compounds of the present invention embraces both cis (*Z*) and trans (*E*) alkene isomers, as well as cis and trans isomers of cyclic hydrocarbon or heterocyclic rings.

[00117] Throughout the specifications, groups and substituents thereof may be chosen to provide stable moieties and compounds.

[00118] The present invention provides compounds that are capable of treating and preventing disorders and diseases associated with the RyR receptors that regulate calcium channel functioning in cells. More particularly, the present invention provides compounds that are capable of treating or preventing a leak in RyR channels. "Disorders and diseases associated with the RyR receptors" means disorders and diseases that can be treated and/or prevented by modulating the RyR receptors that regulate calcium channel functioning in cells. "Disorders and diseases associated with the RyR receptors" include, without limitation, cardiac disorders and diseases, skeletal muscular disorders and diseases, cognitive disorders and diseases, malignant hyperthermia, diabetes, and sudden infant death syndrome. Cardiac disorder and diseases include, but are not limited to, irregular heartbeat disorders and diseases; exercise-induced irregular heartbeat disorders and diseases; sudden cardiac death; exercise-induced sudden cardiac death; congestive heart failure; chronic obstructive pulmonary disease; and high blood pressure. Irregular heartbeat disorders and diseases include and exercise-induced irregular heartbeat disorders and diseases include, but are not limited to, atrial and ventricular arrhythmia; atrial and ventricular fibrillation; atrial and ventricular tachyarrhythmia; atrial and ventricular tachycardia; catecholaminergic polymorphic ventricular tachycardia (CPVT); and exercise-induced variants thereof.

[00119] An important hallmark of heart failure is reduced myocardial contractility (Gwathmey *et al.*, Abnormal intracellular calcium handling in myocardium from patients with end-stage heart failure. *Circ. Res.*, 61:70-76, 1987). In healthy heart muscle, and other striated muscle, calcium-release channels on the sarcoplasmic reticulum (SR), including ryanodine receptors (RyRs), facilitate coupling of the action potential to a muscle cell's contraction (*i.e.*, excitation-contraction (EC) coupling). Contraction is initiated when calcium (Ca^{2+}) is released from the SR into the surrounding cytoplasm. In heart failure, contractile abnormalities result, in part, from alterations in the signaling cascade that allows the cardiac action potential to trigger contraction. In particular, in failing hearts, the

amplitude of the whole-cell Ca^{2+} transient is decreased (Beuckelmann *et al.*, Intracellular calcium handling in isolated ventricular myocytes from patients with terminal heart failure. *Circ.*, 85:1046-55, 1992; Gomez *et al.*, Defective excitation-contraction coupling in experimental cardiac hypertrophy and heart failure. *Science*, 276:800-06, 1997), and the duration prolonged (Beuckelmann *et al.*, Intracellular calcium handling in isolated ventricular myocytes from patients with terminal heart failure. *Circ.*, 85:1046-55, 1992).

[00120] Cardiac arrhythmia, a common feature of heart failure, results in many of the deaths associated with the disease. Atrial fibrillation (AF) is the most common cardiac arrhythmia in humans, and represents a major cause of morbidity and mortality (Chugh *et al.*, Epidemiology and natural history of atrial fibrillation: clinical implications. *J. Am. Coll. Cardiol.*, 37:371-78, 2001; Falk, R.H., Atrial fibrillation. *N. Engl. J. Med.*, 344:1067-78, 2001). Despite AF's clinical importance, the molecular mechanisms underlying this arrhythmia are poorly understood, and treatment options are limited.

[00121] It is well established that structural and electrical remodeling – including shortening of atrial refractoriness, loss of rate-related adaptation of refractoriness (Wijffels *et al.*, Atrial fibrillation begets atrial fibrillation: a study in awake chronically instrumented goats. *Circulation*, 92:1954-68, 1995; Morillo *et al.*, Chronic rapid atrial pacing: structural, functional, and electrophysiological characteristics of a new model of sustained atrial fibrillation. *Circulation*, 91:1588-95, 1995; Elvan *et al.*, Pacing-induced chronic atrial fibrillation impairs sinus node function in dogs: electrophysiological remodeling. *Circulation*, 94:2953-60, 1996; Gaspo *et al.*, Functional mechanisms underlying tachycardia-induced sustained atrial fibrillation in a chronic dog model. *Circulation*, 96:4027-35, 1997), and shortening of the wavelength of re-entrant wavelets – accompany sustained tachycardia (Rensma *et al.*, Length of excitation wave and susceptibility to reentrant atrial arrhythmias in normal conscious dogs. *Circ. Res.*, 62:395-410, 1988). This remodeling is likely important in the development, maintenance and progression of atrial fibrillation.

[00122] Previous studies suggest that calcium handling may play a role in electrical remodeling in atrial fibrillation (Sun *et al.*, Cellular mechanisms of atrial contractile dysfunction caused by sustained atrial tachycardia. *Circulation*, 98:719-27, 1998; Goette *et al.*, Electrical remodeling in atrial fibrillation: time course and mechanisms. *Circulation*, 94:2968-74, 1996; Daoud *et al.*, Effect of verapamil and procainamide on atrial fibrillation-

induced electrical remodeling in humans. *Circulation*, 96:1542-50, 1997; Yu *et al.*, Tachycardia-induced change of atrial refractory period in humans: rate dependency and effects of antiarrhythmic drugs. *Circulation*, 97:2331-37, 1998; Leistad *et al.*, Atrial contractile dysfunction after short-term atrial fibrillation is reduced by verapamil but increased by BAY K8644. *Circulation*, 93:1747-54, 1996; Tieleman *et al.*, Verapamil reduces tachycardia-induced electrical remodeling of the atria. *Circulation*, 95:1945-53, 1997). However, regulation of RyR2 during atrial fibrillations has not previously been reported.

[00123] Approximately 50% of all patients with heart disease die from fatal cardiac arrhythmias. In some cases, a ventricular arrhythmia in the heart may be rapidly fatal – a phenomenon referred to as "sudden cardiac death" (SCD). Fatal ventricular arrhythmias (and SCD) may also occur in young, otherwise-healthy individuals who are not known to have structural heart disease. In fact, ventricular arrhythmia is the most common cause of sudden death in otherwise-healthy individuals.

[00124] Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited disorder in individuals with structurally-normal hearts. It is characterized by stress-induced ventricular tachycardia – a lethal arrhythmia that may cause SCD. In subjects with CPVT, physical exertion and/or stress induce bidirectional and/or polymorphic ventricular tachycardias that lead to SCD in the absence of detectable structural heart disease (Laitinen *et al.*, Mutations of the cardiac ryanodine receptor (RyR2) gene in familial polymorphic ventricular tachycardia. *Circulation*, 103:485-90, 2001; Leenhardt *et al.*, Catecholaminergic polymorphic ventricular tachycardia in children: a 7-year follow-up of 21 patients. *Circulation*, 91:1512-19, 1995; Priori *et al.*, Clinical and molecular characterization of patients with catecholaminergic polymorphic ventricular tachycardia. *Circulation*, 106:69-74, 2002; Priori *et al.*, Mutations in the cardiac ryanodine receptor gene (hRyR2) underlie catecholaminergic polymorphic ventricular tachycardia. *Circulation*, 103:196-200, 2001; Swan *et al.*, Arrhythmic disorder mapped to chromosome 1q42-q43 causes malignant polymorphic ventricular tachycardia in structurally normal hearts. *J. Am. Coll. Cardiol.*, 34:2035-42, 1999).

[00125] CPVT is predominantly inherited in an autosomal-dominant fashion. Individuals with CPVT have ventricular arrhythmias when subjected to exercise, but do not develop arrhythmias at rest. Linkage studies and direct sequencing have identified mutations

in the human RyR2 gene, on chromosome 1q42-q43, in individuals with CPVT (Laitinen *et al.*, Mutations of the cardiac ryanodine receptor (RyR2) gene in familial polymorphic ventricular tachycardia. *Circulation*, 103:485-90, 2001; Priori *et al.*, Mutations in the cardiac ryanodine receptor gene (hRyR2) underlie catecholaminergic polymorphic ventricular tachycardia. *Circulation*, 103:196-200, 2001; Swan *et al.*, Arrhythmic disorder mapped to chromosome 1q42-q43 causes malignant polymorphic ventricular tachycardia in structurally normal hearts. *J. Am. Coll. Cardiol.*, 34:2035-42, 1999).

[00126] There are three types of ryanodine receptors, all of which are highly-related Ca^{2+} channels: RyR1, RyR2, and RyR3. RyR1 is found in skeletal muscle, RyR2 is found in the heart, and RyR3 is located in the brain. The type 2 ryanodine receptor (RyR2) is the major Ca^{2+} -release channel required for EC coupling and muscle contraction in cardiac striated muscle.

[00127] RyR2 channels are packed into dense arrays in specialized regions of the SR that release intracellular stores of Ca^{2+} , and thereby trigger muscle contraction (Marx *et al.*, Coupled gating between individual skeletal muscle Ca^{2+} release channels (ryanodine receptors). *Science*, 281:818-21, 1998). During EC coupling, depolarization of the cardiac-muscle cell membrane, in phase zero of the action potential, activates voltage-gated Ca^{2+} channels. In turn, Ca^{2+} influx through these channels initiates Ca^{2+} release from the SR via RyR2, in a process known as Ca^{2+} -induced Ca^{2+} release (Fabiato, A., Calcium-induced release of calcium from the cardiac sarcoplasmic reticulum. *Am. J. Physiol.*, 245:C1-C14, 1983; Nabauer *et al.*, Regulation of calcium release is gated by calcium current, not gating charge, in cardiac myocytes. *Science*, 244:800-03, 1989). The RyR2-mediated, Ca^{2+} -induced Ca^{2+} release then activates the contractile proteins which are responsible for cardiac muscle contraction.

[00128] RyR2 is a protein complex comprising four 565,000-dalton RyR2 polypeptides in association with four 12,000-dalton FK506 binding proteins (FKBPs), specifically FKBP12.6 (calstabin). FKBPs are *cis-trans* peptidyl-prolyl isomerases that are widely expressed and serve a variety of cellular functions (Marks, A.R., Cellular functions of immunophilins. *Physiol. Rev.*, 76:631-49, 1996). FKBP12 proteins are tightly bound to, and regulate the function of, the skeletal ryanodine receptor, RyR1 (Brillantes *et al.*, Stabilization of calcium release channel (ryanodine receptor) function by FK506-binding protein. *Cell*, 77:513-23, 1994; Jayaraman *et al.*, FK506 binding protein associated with the

calcium release channel (ryanodine receptor). *J. Biol. Chem.*, 267:9474-77, 1992); the cardiac ryanodine receptor, RyR2 (Kaftan *et al.*, Effects of rapamycin on ryanodine receptor/Ca(2+)-release channels from cardiac muscle. *Circ. Res.*, 78:990-97, 1996); a related intracellular Ca²⁺-release channel, known as the type 1 inositol 1,4,5-trisphosphate receptor (IP3R1) (Cameron *et al.*, FKBP12 binds the inositol 1,4,5-trisphosphate receptor at leucine-proline (1400-1401) and anchors calcineurin to this FK506-like domain. *J. Biol. Chem.*, 272:27582-88, 1997); and the type I transforming growth factor β (TGF β) receptor (T β RI) (Chen *et al.*, Mechanism of TGF β receptor inhibition by FKBP12. *EMBO J.*, 16:3866-76, 1997). FKBP12.6 binds to the RyR2 channel (one molecule per RyR2 subunit), stabilizes RyR2-channel function (Brillantes *et al.*, Stabilization of calcium release channel (ryanodine receptor) function by FK506-binding protein. *Cell*, 77:513-23, 1994), and facilitates coupled gating between neighboring RyR2 channels (Marx *et al.*, Coupled gating between individual skeletal muscle Ca²⁺ release channels (ryanodine receptors). *Science*, 281:818-21, 1998), thereby preventing aberrant activation of the channel during the resting phase of the cardiac cycle.

[00129] Phosphorylation of cardiac RyR2 by protein kinase (PKA) is an important part of the "fight or flight" response that increases cardiac EC coupling gain by augmenting the amount of Ca²⁺ released for a given trigger (Marks, A.R., Cardiac intracellular calcium release channels: role in heart failure. *Circ. Res.*, 87:8-11, 2000). This signaling pathway provides a mechanism by which activation of the sympathetic nervous system, in response to stress, results in increased cardiac output required to meet the metabolic demands of the stress responses. Upon binding of catecholamines, β 1- and β 2-adrenergic receptors activate adenylyl cyclase *via* a stimulatory G-protein, G α_s . Adenylyl cyclase increases intracellular cAMP levels, which activate the cAMP-dependent PKA. PKA phosphorylation of RyR2 increases the open probability of the channel by dissociating calstabin2 (FKBP12.6) from the channel complex. This, in turn, increases the sensitivity of RyR2 to Ca²⁺-dependent activation (Hain *et al.*, Phosphorylation modulates the function of the calcium release channel of sarcoplasmic reticulum from cardiac muscle. *J. Biol. Chem.*, 270:2074-81, 1995; Valdivia *et al.*, Rapid adaptation of cardiac ryanodine receptors: modulation by Mg²⁺ and phosphorylation. *Science*, 267:1997-2000, 1995; Marx *et al.*, PKA phosphorylation dissociates FKBP12.6 from the calcium release channel (ryanodine receptor): defective regulation in failing hearts. *Cell*, 101:365-76, 2000).

[00130] Failing hearts (*e.g.*, in patients with heart failure and in animal models of heart failure) are characterized by a maladaptive response that includes chronic hyperadrenergic stimulation (Bristow *et al.*, Decreased catecholamine sensitivity and beta-adrenergic-receptor density in failing human hearts. *N. Engl. J. Med.*, 307:205-11, 1982). The pathogenic significance of this stimulation in heart failure is supported by therapeutic strategies that decrease beta-adrenergic stimulation and left ventricular myocardial wall stress, and potentially reverse ventricular remodeling (Barbone *et al.*, Comparison of right and left ventricular responses to left ventricular assist device support in patients with severe heart failure: a primary role of mechanical unloading underlying reverse remodeling. *Circulation*, 104:670-75, 2001; Eichhorn and Bristow, Medical therapy can improve the biological properties of the chronically failing heart. A new era in the treatment of heart failure. *Circulation*, 94:2285-96, 1996). In heart failure, chronic beta-adrenergic stimulation is associated with the activation of beta-adrenergic receptors in the heart, which, through coupling with G-proteins, activate adenylyl cyclase and thereby increase intracellular cAMP concentration. cAMP activates cAMP-dependent PKA, which has been shown to induce hyperphosphorylation of RyR2. Thus, chronic heart failure is a chronic hyperadrenergic state (Chidsey *et al.*, Augmentation of plasma norepinephrine response to exercise in patients with congestive heart failure. *N. Engl. J. Med.*, 267:650, 1962) which results in several pathologic consequences, including PKA hyperphosphorylation of RyR2 (Marx *et al.*, PKA phosphorylation dissociates FKBP12.6 from the calcium release channel (ryanodine receptor): defective regulation in failing hearts. *Cell*, 101:365-76, 2000).

[00131] The PKA hyperphosphorylation of RyR2 has been proposed as a factor contributing to depressed contractile function and arrhythmogenesis in heart failure (Marks *et al.*, Progression of heart failure: is protein kinase a hyperphosphorylation of the ryanodine receptor a contributing factor? *Circulation*, 105:272-75, 2002; Marx *et al.*, PKA phosphorylation dissociates FKBP12.6 from the calcium release channel (ryanodine receptor): defective regulation in failing hearts. *Cell*, 101:365-76, 2000). Consistent with this hypothesis, PKA hyperphosphorylation of RyR2 in failing hearts has been demonstrated *in vivo*, both in animal models and in patients with heart failure undergoing cardiac transplantation (Antos *et al.*, Dilated cardiomyopathy and sudden death resulting from constitutive activation of protein kinase A. *Circ. Res.*, 89:997-1004, 2001; Marx *et al.*, PKA phosphorylation dissociates FKBP12.6 from the calcium release channel (ryanodine receptor): defective regulation in failing hearts. *Cell*, 101:365-76, 2000; Ono *et al.*, Altered

interaction of FKBP12.6 with ryanodine receptor as a cause of abnormal Ca^{2+} release in heart failure. *Cardiovasc. Res.*, 48:323-31, 2000; Reiken *et al.*, Beta-adrenergic receptor blockers restore cardiac calcium release channel (ryanodine receptor) structure and function in heart failure. *Circulation*, 104:2843-48, 2001; Semsarian *et al.*, The L-type calcium channel inhibitor diltiazem prevents cardiomyopathy in a mouse model. *J. Clin. Invest.*, 109:1013-20, 2002; Yano *et al.*, Altered stoichiometry of FKBP12.6 versus ryanodine receptor as a cause of abnormal Ca^{2+} leak through ryanodine receptor in heart failure. *Circulation*, 102:2131-36, 2000).

[00132] In failing hearts, the hyperphosphorylation of RyR2 by PKA induces the dissociation of the regulatory FKBP12.6 subunit from the RyR2 channel (Marx *et al.*, PKA phosphorylation dissociates FKBP12.6 from the calcium release channel (ryanodine receptor): defective regulation in failing hearts. *Cell*, 101:365-76, 2000). This causes marked changes in the biophysical properties of the RyR2 channel. Such changes are evidenced by increased open probability (P_o), due to an increased sensitivity to Ca^{2+} -dependent activation (Brillantes *et al.*, Stabilization of calcium release channel (ryanodine receptor) function by FK506-binding protein. *Cell*, 77:513-23, 1994; Kaftan *et al.*, Effects of rapamycin on ryanodine receptor / Ca^{2+} -release channels from cardiac muscle. *Circ. Res.*, 78:990-97, 1996); destabilization of the channel, resulting in subconductance states; and impaired coupled gating of the channels, resulting in defective EC coupling and cardiac dysfunction (Marx *et al.*, Coupled gating between individual skeletal muscle Ca^{2+} release channels (ryanodine receptors). *Science*, 281:818-21, 1998). Thus, PKA-hyperphosphorylated RyR2 is very sensitive to low-level Ca^{2+} stimulation, and this manifests itself as an SR Ca^{2+} leak through the hyperphosphorylated channel.

[00133] The maladaptive response to stress in heart failure results in depletion of FKBP12.6 from the channel macromolecular complex. This leads to a shift to the left in the sensitivity of RyR2 to Ca^{2+} -induced Ca^{2+} release, resulting in channels that are more active at low-to-moderate $[\text{Ca}^{2+}]$ (Marx *et al.*, PKA phosphorylation dissociates FKBP12.6 from the calcium release channel (ryanodine receptor): defective regulation in failing hearts. *Cell*, 101:365-76, 2000; Yamamoto *et al.*, Abnormal Ca^{2+} release from cardiac sarcoplasmic reticulum in tachycardia-induced heart failure. *Cardiovasc. Res.*, 44:146-55, 1999; Yano *et al.*, Altered stoichiometry of FKBP12.6 versus ryanodine receptor as a cause of abnormal Ca^{2+} leak through ryanodine receptor in heart failure. *Circulation*, 102:2131-36, 2000).

Over time, the increased "leak" through RyR2 results in resetting of the SR Ca^{2+} content to a lower level, which in turn reduces EC coupling gain and contributes to impaired systolic contractility (Marx *et al.*, PKA phosphorylation dissociates FKBP12.6 from the calcium release channel (ryanodine receptor): defective regulation in failing hearts. *Cell*, 101:365-76, 2000).

[00134] Additionally, a subpopulation of RyR2 that are particularly "leaky" can release SR Ca^{2+} during the resting phase of the cardiac cycle, diastole. This results in depolarizations of the cardiomyocyte membrane known as delayed after-depolarizations (DADs), which are known to trigger fatal ventricular cardiac arrhythmias (Wehrens *et al.*, FKBP12.6 deficiency and defective calcium release channel (ryanodine receptor) function linked to exercise-induced sudden cardiac death. *Cell*, 113:829-40, 2003).

[00135] In structurally-normal hearts, a similar phenomenon may be at work. Specifically, it is known that exercise and stress induce the release of catecholamines that activate beta-adrenergic receptors in the heart. Activation of the beta-adrenergic receptors leads to hyperphosphorylation of RyR2 channels. Evidence also suggests that the hyperphosphorylation of RyR2 resulting from beta-adrenergic-receptor activation renders mutated RyR2 channels more likely to open in the relaxation phase of the cardiac cycle, increasing the likelihood of arrhythmias.

[00136] Cardiac arrhythmias are known to be associated with SR Ca^{2+} leaks in structurally-normal hearts. In these cases, the most common mechanism for induction and maintenance of ventricular tachycardia is abnormal automaticity. One form of abnormal automaticity, known as triggered arrhythmia, is associated with aberrant release of SR Ca^{2+} , which initiates DADs (Fozzard, H.A., Afterdepolarizations and triggered activity. *Basic Res. Cardiol.*, 87:105-13, 1992; Wit and Rosen, Pathophysiologic mechanisms of cardiac arrhythmias. *Am. Heart J.*, 106:798-811, 1983). DADs are abnormal depolarizations in cardiomyocytes that occur after repolarization of a cardiac action potential. The molecular basis for the abnormal SR Ca^{2+} release that results in DADs has not been fully elucidated. However, DADs are known to be blocked by ryanodine, providing evidence that RyR2 may play a key role in the pathogenesis of this aberrant Ca^{2+} release (Marban *et al.*, Mechanisms of arrhythmogenic delayed and early afterdepolarizations in ferret ventricular muscle. *J. Clin. Invest.*, 78:1185-92, 1986; Song

and Belardinelli, ATP promotes development of afterdepolarizations and triggered activity in cardiac myocytes. *Am. J. Physiol.*, 267:H2005-11, 1994).

[00137] Arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C) is a relatively newly recognized disease. Clinical symptoms of patients with ARVC include ventricular tachycardia of left bundle-branch block morphology. An enlarged right ventricle due to fibrofatty infiltration of the right ventricular free wall and a familial association are also noted. Noninvasive tests, including ECG, echocardiography, and signal-averaged ECG can be used to diagnose symptoms associated with the disease. Some studies also suggest that MRI may also be used for diagnostic purposes.

[00138] The term arrhythmogenic right ventricular cardiomyopathy (ARVC) encompasses arrhythmogenic right ventricular dysplasia (ARVD) and several other clinical entities, some of them known by a different name. The term *right ventricular dysplasia* was chosen because the right ventricle of some patients undergoing cardiac surgery was covered by fat. The remaining myocardium was present only in the subendocardium. This striking feature, which suggested the replacement of myocardium by fat, was strengthened by microscopic examination that showed strands of cardiomyocytes bordered by or sometimes embedded in fibrosis or adipose tissue. It was suspected that myocardial replacement by fat and fibrous tissue started early in life, possibly in the embryo. The role of apoptosis was also demonstrated as one of the factors contributing to myocardial remodeling in ARVD. Whether fatty infiltration of the right ventricle has to be considered "per se" a sufficient morphologic hallmark of arrhythmogenic right ventricular cardiomyopathy (ARVC) is still a source of controversy; ARVC is considered distinct from both fatty infiltration of the right ventricle and adipositas cordis (Basso et al. *Cardiovascular Pathology* 2005, 14(1):37-41).

[00139] Arrhythmogenic right ventricular cardiomyopathy (ARVC) can be characterized by progressive fibro-fatty replacement of right ventricular myocardium. ARVC is described as non-inflammatory, non-coronary disorder associated with arrhythmias, heart failure and sudden death due to functional exclusion of the right ventricle. Molecular genetic studies have identified nine different loci associated with ARVC; accordingly each locus is implicated in a type of ARVC (ARVC1-ARVC9). A more accurate classification of the various subgroups of these right ventricular cardiomyopathies can be made on the basis of genetic and molecular characteristics, in addition to clinical symptoms and pathological examination of cardiac tissues. So far

mutations in five genes have been identified as containing changes that result in the pathogenic characteristics of ARVC.

[00140] Cardiac ryanodine receptor (RyR2), which regulates intra-cellular Ca^{2+} concentration by releasing Ca^{2+} reserves from the sarcoplasmic reticulum (SR), was the first gene implicated as an underlying cause of ARVC. One of the genetic changes in RyR2 gene was identified in a group of patients in Italy with a familial dominant form of exercise-induced ventricular arrhythmia (Tiso et al. 2001, *Human Molecular Genetics*, 10:189-194). Three other genes that were implicated in ARVC, plakoglobin (Naxos disease), desmoplakin (ARVC8) and plakophilin (ARVC9), have prompted the speculation that ARVC is primarily a disease of desmosomes. For example, Naxos disease is an inherited condition with a recessive form of transmission which is associated with clinically obvious ectodermic dysplasia (keratoderma, woolly hair) and has the typical histological pattern of ARVD. This association between the ectodermic dysplasia and the histological pattern of ARVD, led to the identification of a nonsense mutation in a gene encoding plakoglobin. Mutation in this gene can explain both ectodermal manifestations and cardiac dysplasia (McKoy et al. *Lance* 2000, 355:2119-2124).

[00141] In another form of the disease identified in Ecuador, where patients present clinically with idiopathic dilated cardiomyopathy without fatty infiltration, a recessive mutation in the gene encoding desmoplakin, which is in the category of cell-cell adhesion factors, was identified (Norgett et al., *Human Molecular Genetics* 2000, 9:2761-2766). Mutation in the gene encoding desmoplakin was identified in the Veneto region of Italy in a familial dominant form of ARVD (Rampazzo et al. 2002, *American Journal of Human Genetics*, 71:1200-1206).

[00142] However, the identification of TGFbeta-3 for ARVC1 indicates that ARVC is not a diseases of desmosomes. Ryanodide-2 receptor, TGFbeta-3, plakoglobin, desmoplakin and plakophilin have diverse cellular roles in cardiac morphogenesis, which suggests that there is a disruption of signal-transduction mechanism which causes the clinical condition defined as ARVC. Furthermore, discovery of apoptotic cells during autopsy of the right ventricular myocardium of ARVC patients indicates a common pathway that underlies the cause of different types of ARVCs. This pathway appears to be specific to the right ventricular myocardium and involves desmosomal plaque proteins,

growth factors and Ca^{2+} receptors (Dokuparti et al. 2005, *Journal Of Human Genetics*, 50(8):375-81.)

[00143] Because of the complex ethiopathology of ARVC, standardized criteria for diagnosis of ARVC have been proposed. These criteria include structural, histological, electrocardiographic, arrhythmic and genetic factors (Dokuparti et al. 2005, *Journal Of Human Genetics*, 50(8):375-81.) These criteria include: family history, including familial disease confirmed by autopsy or surgery, family history of premature sudden death; ECG abnormalities including, depolarization/repolarization or conduction abnormalities; arrhythmias, including sustained or non-sustained ventricular tachycardia, ventricular extra systoles; structural alterations, including dilation and reduction of right ventricular ejection fraction with mild ventricular involvement, localized ventricular aneurysms, dilation of the right ventricle; changes in tissue characteristics of the ventricle walls, including fibro fatty replacement of myocardium detected in an endomyocardial biopsy, or changes in the tissue characteristic detected by echocardiography, angiography, magnetic resonance imaging, or radionuclide scintigraphy.

[00144] Another form of ventricular arrhythmia frequently observed in young individuals is right ventricular outflow tract (RVOT) Ventricular Tachycardia (VT). Some patients with benign ventricular extra systoles arising from the RVOT may develop rapid VT degenerating into ventricular fibrillation. The autopsy results in at least one case of RVOT demonstrate the presence of fat and fibrosis in surviving fibers, signs of inflammation in the infundibular area, These autopsy results suggest a localized form of ARVD.

[00145] Use of antiarrhythmic drugs and Implantable Cardiac Defibrilators (ICDs) has been proposed for the management of ventricular tacharrhythmias associated with ARVC (Wichter et al. 1992, *Circulation*, 86:29-37; Wichter et al. 2004, *Circulation*, 109:1503-1508; Fontaine et al. 2004, *Circulation*, 109:1445-1447).

[00146] Non-human Animals

[00147] It is notable that clinical manifestations of cardiac conditions are not limited to human patients. Non-human animals are also afflicted with cardiac problems that are reminiscent of human cardiac conditions, including various arrhythmias and cardiomyopathies that can lead to SCD.

[00148] A clinically relevant feline cardiomyopathy closely mimicking human ARVD/C was described in the domestic cat. A study identified and analyzed twelve domestic cats which presented with clinical features of ARVC. Eight of the twelve cats died of progressive right-sided congestive heart failure (CHF), while two were ultimately euthenized despite aggressive medical management with diuretics, ACE inhibitors, and digitalis. The ARVC cats frequently showed ECG abnormalities similar to those in patients with this disease, including supraventricular tachyarrhythmias (particularly atrial fibrillation), complex ventricular ectopy including ventricular tachycardia, and major conduction abnormalities (Fox, P. R., B. J. Maron, et al. (2000). "Spontaneously occurring arrhythmogenic right ventricular cardiomyopathy in the domestic cat: A new animal model similar to the human disease." *Circulation* 102(15): 1863-70).

[00149] A family of Maine coon cats with an inherited form of hypertrophic cardiomyopathy (HCM) has been identified. Familial hypertrophic cardiomyopathy (FHCM) is a common hereditary human disease caused by mutations in 7 different genes encoding proteins of the myofibrillar apparatus. HCM in these animals is inherited as an autosomal dominant trait and is recognized as a common cause of heart failure, sudden death, and systemic thromboembolism. HCM here is characterized by gross left ventricular (LV) wall thickening, dynamic left ventricular outflow tract (LVOT) obstruction, myocardial fiber disarray, small coronary artery disease, and myocardial fibrosis. Five of the 16 affected cats died from sudden cardiac death (Kittleson, M. D., K. M. Meurs, et al. (1999). "Familial hypertrophic cardiomyopathy in Maine coon cats: an animal model of human disease." *Circulation* 99(24): 3172-80).

[00150] The gene responsible for the HCM phenotype in these cats has been identified as the *MYBPC3* gene. *MYBPC3* was chosen as a candidate gene in this model due to a reduction in the concentrations of its protein product in myocardium from affected cats. The mutations alters protein conformation of the gene product and results in sarcomeric disorganization (Meurs, K. M., X. Sanchez, et al. (2005). "A cardiac myosin binding protein C mutation in the Maine Coon cat with familial hypertrophic cardiomyopathy." *Hum Mol Genet* 14(23): 3587-93).

[00151] In horses, including racehorses, atrial fibrillation (AF), atrial premature contractions APCs or ventricular premature contractions (VPCs) occasionally occur during or shortly after the stress of a race. Cardiopathological studies on five apparently normal

racehorses, which died during or shortly after intensive training exercise, indicated sudden cardiac death (SCD) due to arrhythmias. Myocardial fibrosis found in the horse's cardiac conduction system may have induced VPCs, which rapidly degenerated into ventricular fibrillation (VF) and SCD. Electrocardiography recordings on one of the animals confirmed the presence of an arrhythmia (Kiryu, K., N. Machida, et al. (1999). "Pathologic and electrocardiographic findings in sudden cardiac death in racehorses." *J Vet Med Sci* 61(8): 921-8).

[00152] An inbred strain of rats called spontaneously hypertensive rats (SHR) have been used to study hypertension. In the SHR rats, LV hypertrophy is present from an early age when blood pressure is within the "normotensive" range. In addition, certain treatments that normally cause a reduction in blood pressure have quite different effects on SHR heart size (Innes, B. A., M. G. McLaughlin, et al. (1998). "Independent genetic susceptibility to cardiac hypertrophy in inherited hypertension." *Hypertension* 31(3): 741-6).

[00153] Among eight calves infected with *Strongyloides papillosus*, six animals suffered from SCD within 11 to 17 days after infection. Sinus tachycardia was observed starting at about 6 days before death along with gradually increasing heart rates until death. Starting at about 1 or 2 days before death, various patterns of tachyarrhythmia and bradyarrhythmia had been observed among patterns of sinus tachycardia. Arrhythmias included serious ventricular premature beats, paroxysmal ventricular tachycardia, and complete atrioventricular block. In all of the cases, the terminal pattern observed was ventricular arrhythmias consisting of serial ventricular tachycardia, flutter and fibrillation followed by respiratory arrest (Tsuji, N., T. Itabisashi, et al. (1992). "Sudden cardiac death in calves with experimental heavy infection of *Strongyloides papillosus*." *J Vet Med Sci* 54(6): 1137-43).

[00154] Another similar study showed that lambs inoculated with the parasitic females of *S. papillosus* developed continuous sinus tachycardia and died of sudden cardiac arrest by ventricular fibrillation (Nakamura, Y., N. Tsuji, et al. (1994). "Parasitic females of *Strongyloides papillosus* as a pathogenetic stage for sudden cardiac death in infected lambs." *J Vet Med Sci* 56(4): 723-7).

[00155] Young and fast-growing Broiler chickens have a high risk of developing sudden death syndrome (SDS), with about 60 to 80% of those affected being males. Since

mortality peaks from 1 to 3 weeks of age, the age at which the rate of feed conversion is greatest, it has been suggested that the ventricular fibrillations leading to SDS are caused by a metabolic disease. This disorder, however, has relatively low prevalence amongst chickens making genetic studies difficult to conduct (Moghadam, H. K., I. McMillan, et al. (2005). "Heritability of sudden death syndrome and its associated correlations to ascites and body weight in broilers." *Br Poult Sci* 46(1): 54-7).

[00156] A variety of factors such as nutrition, genetics and environmental factors can affect the incidence of SDS, making most modern Broiler chicken strains susceptible. The incidence of SDS appears to increase appreciably as flock body weight increases. There also appears to be a positive genetic correlation between SDS and AS (ascites – fluid in the abdomen) disorder (Moghadam, H. K., I. McMillan, et al. (2005). "Heritability of sudden death syndrome and its associated correlations to ascites and body weight in broilers." *Br Poult Sci* 46(1): 54-7).

[00157] Comparison studies of skeletal muscle SR calcium regulation in Broiler and Leghorn chickens were conducted to determine the cause of SDS in the Broiler strains. Results showed that Broiler chickens had significantly lower calcium transport rates and transport efficiencies compared to the Leghorn chickens. The mechanism of calcium transport in Broiler chickens was also more energy-consuming than that of the Leghorn chickens. As in porcine malignant hyperthermia, weaker calcium regulation might lead to hyperactivation of skeletal muscle, followed by elevated lactic acid concentrations and cardiovascular failure (Reiner, G., J. Hartmann, et al. (1995). "Skeletal muscle sarcoplasmic calcium regulation and sudden death syndrome in chickens." *Br Poult Sci* 36(4): 667-75).

[00158] Pigs marginally deficient in magnesium (Mg) and fed diets high in manganese (Mn) died suddenly with signs of sudden cardiac death (SCD). The decreased Mg content of cardiac muscle increased the risk of mortality from sudden-death ischemic heart disease; the presence of Mn exacerbated the effects of the hypomagneseia. Magnesium deficiency was reported to have modulated intracellular myocardial calcium by blocking efflux through intracellular calcium channels (Miller, K. B., S. M. Newman, Jr., et al. (2004). "Manganese alters mitochondrial integrity in the hearts of swine marginally deficient in magnesium." *Biofactors* 20(2): 85-96).

[00159] Modern methods of swine breeding including large-scale blood sampling, one-sided breeding (to get a high lean meat content), and artificial growth hormones have increased the number of exertional myopathies in pigs. In addition, due to modern housing systems, pigs grow up in an environment without any stimulus, which results in emotional stress when the pigs are suddenly placed in close proximity with humans. Therefore, a routine operation such as taking blood can lead to lactacidosis and sometimes a circulatory insufficiency. The metabolic acidosis leading to SCD is often strengthened by pneumonia in fattening herds as well as faulty husbandry conditions that prevent compensation by respiration. The exertional myopathy is not only characterized by sudden death but also by poor meat quality due to pale, soft, and exudative (PSE) meat (von Altrock, A. and K. von Holleben (1999). "[Sudden deaths in taking blood samples from fattening swine herds--experiences from practice]." *Berl Munch Tierarztl Wochenschr* 112(3): 86-90). Unless inexpensive pharmacologic treatments are developed, it will be desirable from the point of view of animal welfare and consumers that breeding methods be changed.

[00160] Knock-in mice containing the R4496C mutation in cardiac ryanodine receptor were more likely to develop VT and VF (5/14) in response to caffeine and/or adrenergic stimulation compared to WT (0/14). This mutation mimics a mutation found in humans who display CPVT phenotypes. Results from tests with beta blockers showed that beta adrenergic stimulation seems ineffective in preventing the life-threatening arrhythmias in these mice (Cerrone, M., B. Colombi, et al. (2005). "Bidirectional ventricular tachycardia and fibrillation elicited in a knock-in mouse model carrier of a mutation in the cardiac ryanodine receptor." *Circ Res* 96(10): e77-82).

[00161] Genetically engineered mice with cardiac-restricted knockout of Connexin43, the major cardiac gap junctional protein, uniformly develop sudden cardiac death, although a detailed electrophysiological understanding of their profound arrhythmic propensity is unclear. (Morley, Danik et al. 2005) Similar mutations in human ankyrin-B causes dominantly inherited type 4 long-QT cardiac arrhythmia by disrupting critical EC-coupling protein connections to T-tubules. Ankyrin-B mutations lead to an increase in $[Ca^{2+}]_i$ transient under these conditions implying that the amount of calcium in the sarcoplasmic reticulum is elevated. The altered calcium signaling provides a rationale for the arrhythmia (Mohler, P. J., J. J. Schott, et al. (2003). "Ankyrin-B mutation causes type 4 long-QT cardiac arrhythmia and sudden cardiac death." *Nature* 421(6923): 634-9).

[00162] While humans, rats, dogs, and cats with prolonged action potential duration (APD) show signs of prominent I_{to} downregulation, this is absent in guinea pig ventricular myocytes. Guinea pig ventricles do not express I_{to} and therefore, APD prolongation in these animals is unrelated to K^+ currents. Therefore, an unknown alternative mechanism for action potential alteration in cardiac hypertrophy and failure exists in these animals (Ahmmed, G. U., P. H. Dong, et al. (2000). "Changes in Ca^{2+} cycling proteins underlie cardiac action potential prolongation in a pressure-overloaded guinea pig model with cardiac hypertrophy and failure." *Circ Res* 86(5): 558-70).

[00163] The Syrian cardiomyopathic hamster (CMH) is known to develop a genetically determined cardiomyopathy, with progressive development of CHF. While several sites of cellular dysfunction including defects in mitochondria, sarcoplasmic reticulum, myofibrils, and sarcolemma have been reported in these animals, the primary defect initiating the chain of events has not been identified. Current experimental data has shown that despite normal L-type calcium channel function, major abnormalities in calcium homeostasis exist. The pathogenesis to CHF is believed to be caused by dysfunctions in intracellular calcium handling (Sen, L. Y., M. O'Neill, et al. (1990). "Inotropic and calcium kinetic effects of calcium channel agonist and antagonist in isolated cardiac myocytes from cardiomyopathic hamsters." *Circ Res* 67(3): 599-608).

[00164] Various canines are also afflicted with cardiac problems and shown symptoms of cardiac arrhythmias, including inherited arrhythmias. Canines of large breeds are particularly predisposed to developing cardiac arrhythmogenic conditions. A study of a family of German Shepherd dogs, identified that there is a window of vulnerability to death between 15 and 76 weeks of age. In most cases, before death, clinical signs are absent, laboratory data appear normal without any obvious abnormalities, and echocardiographic parameters do not support obvious structural or functional anomalies. Death usually occurs during sleep, in the early morning hours, when a ventricular tachycardia likely degenerates into ventricular fibrillation. These German shepherd dogs display inherited ventricular ectopic activity. The arrhythmias in these animals are pause dependent but are not associated with a prolonged QT interval, and are initiated by early afterdepolarization-induced triggered activity in Purkinje fibers. Several observations (e.g. reduced cell capacitance, reduced I_{to} current density, regional paucity of sympathetic nerve fibers) suggest an error in the normal development of the heart, possibly involving ion channels.

Initial pedigree analyses have revealed that the possibility of a sex-linked or autosomal dominant trait is unlikely (Moise, N. S. (1999). "Inherited arrhythmias in the dog: potential experimental models of cardiac disease." *Cardiovasc Res* 44(1): 37-46).

[00165] Studies on a family of German shepherd dogs with inherited risks of SCD have found that reduced sympathetic innervation in the antero-septal left ventricle may contribute to arrhythmogenesis. Differences in a number of repolarizing K(+) currents have been identified in this model, but I(Ca,L) has not yet been studied (Protas, L., E. A. Sosunov, et al. (2005). "Regional dispersion of L-type calcium current in ventricular myocytes of German shepherd dogs with lethal cardiac arrhythmias." *Heart Rhythm* 2(2): 172-6).

[00166] Sick sinus syndrome (SSS) with familial occurrence has been reported in the Miniature Schnauzer, West Highland White terriers, and Dachshunds. Female middle-aged (e.g. 6–12 years) dogs are usually presented with syncope, caused by prolonged sinus pauses. While some dogs develop inappropriate sinus bradycardia, others collapse after pauses of 8 seconds or longer, possibly due to the development of supraventricular tachycardia. Commonly affected dogs have coexisting mitral valve incompetence which usually leads to chronic heart failure (CHF) within 5 years irregardless of whether they develop tachycardia (Moise, N. S. (1999). "Inherited arrhythmias in the dog: potential experimental models of cardiac disease." *Cardiovasc Res* 44(1): 37-46).

[00167] A family of English Springer Spaniels has been identified with an inherited form of atrial standstill. This disease is characterized by a bradycardia of junctional escape complexes and no identifiable P waves. Affected animals are usually young adults (e.g. 1 to 3 years of age) whose clinical signs include syncope, lethargy or congestive heart failure (Moise, N. S. (1999). "Inherited arrhythmias in the dog: potential experimental models of cardiac disease." *Cardiovasc Res* 44(1): 37-46).

[00168] Male Golden Retrievers who inherit muscular dystrophy (an X-linked trait) develop severe forms of cardiac disease due to the absence of Dystrophin, an intracellular plasma membrane stabilizing protein. Echocardiography reveals distinctive hyperechoic lesions that correspond to calcified myocardium and surrounding dense connective tissue. Approximately a third of the affected dogs develop polymorphic ventricular arrhythmias

(Moise, N. S. (1999). "Inherited arrhythmias in the dog: potential experimental models of cardiac disease." *Cardiovasc Res* 44(1): 37-46).

[00169] In the USA and Canada, almost 50% of Doberman Pinscher dogs develop dilated cardiomyopathy (DCM), followed by smaller incidences in Newfoundland dogs and Cocker spaniels. Atrial fibrillation is the most common arrhythmia associated with DCM in these dogs. Polymorphic ventricular arrhythmias are common causes of SCD in Doberman Pinschers with DCM, although they may be the result of a bradycardia (Moise, N. S. (1999). "Inherited arrhythmias in the dog: potential experimental models of cardiac disease." *Cardiovasc Res* 44(1): 37-46).

[00170] Other canine breeds at risk of sudden death due to cardiac problems include but are not limited to canines from the breeds of Newfoundland, Cocker Spaniel, Grate Dane, Irish Wolfhound, Afghan Hound, and Saluki. Cardiac problems which lead to sudden death in canines can be associated with cardiac arrhythmias, e.g., ventricular and atrial tachycardia; atrial arrhythmia, including atrial tachyarrhythmia and atrial fibrillation (both sustained and non-sustained); ventricular arrhythmia, including ventricular tachyarrhythmia, ventricular fibrillation; and stress or exercise-induced cardiac arrhythmia, catecholaminergic polymorphic ventricular tachycardia (CPVT), arrhythmogenic right ventricular cardiomyopathy (ARVD/C), hyperthrophic cardiomyopathy, dilated cardiomyopathy, restrictive cardiomyopathy, sick sinus syndrome, atrial standstill sinus tachycardia, and/or stress- or exercise-induced sudden cardiac death.

[00171] Clinical manifestation and cardiac pathology associated with ARVC have been found in canines. Arrhythmogenic right ventricular dysplasia (ARVD) has been diagnosed in a female Labrador dog, and autopsy findings very similar to those described in ARVD were reported in a Siberian Husky as well (Danieli, G. A. and A. Rampazzo (2002). "Genetics of arrhythmogenic right ventricular cardiomyopathy." *Curr Opin Cardiol* 17(3): 218-21). Canines of the breed Boxer are known for their particular predisposition to ventricular arrhythmias and SCD. A family of Boxer dogs has been shown to transmit an autosomal dominant trait of ARVC as well. Of 23 diagnosed boxer dogs, 9/ 23 died suddenly and unexpectedly: 3 during vigorous exercise, 4 during leisurely walking, 2 while sleeping. Of the 9 dogs that died suddenly, 1 had sustained VT, 2 had nonsustained VT with PVCs, and 3 had PVCs alone. ARVC boxer dogs did not differ significantly from controls with regard to heart weight, RV wall thickness, or LV thickness. All 23 boxer dogs

displayed histopathological lesions that closely resembled those characteristic of human patients with ARVC. Myocarditis was conspicuously present in all ARVC dogs with sudden death, suggesting that myocardial inflammation plays a role in arrhythmogenesis (Basso, C., P. R. Fox, et al. (2004). "Arrhythmogenic right ventricular cardiomyopathy causing sudden cardiac death in boxer dogs: a new animal model of human disease." *Circulation* **109**(9): 1180-5). The dogs developed arrhythmias in adulthood (2 and 8 years of age) usually after multiple episodes of syncope (Moise, N. S. (1999). "Inherited arrhythmias in the dog: potential experimental models of cardiac disease." *Cardiovasc Res* **44**(1): 37-46).

[00172] Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a common cardiac affliction in the Boxer dog, and is characterized by ventricular arrhythmias and sudden death. In humans, certain forms of ARVC are associated with abnormalities of the cardiac ryanodine receptor (RyR2) and its associated regulatory molecule, calstabin2 (also called FKBP 12.6). In cardiac myocytes, RyR2 is located on the sarcoplasmic reticulum (SR) and controls release of calcium from SR stores into the cytosol during excitation-contraction coupling. Thus, RyR2 channels are open during systole and closed during diastole. Binding of calstabin2 to the tetrameric RyR2 molecule stabilizes the closed state of RyR2 preventing calcium "leak" during diastole. Loss of calstabin2 binding through decreased calstabin2 transcription or sympathetically-mediated PKA hyperphosphorylation of RyR2 ostensibly causes diastolic "leak" of calcium into the cytosol, which can trigger arrhythmias and reduce efficiency of calcium cycling for myocardial contraction. Thus RyR2-calstabin2 complex plays a critical role in the development of arrhythmias.

[00173] Mutations affecting calstabin2 binding to the ryanodine receptor/calcium release channel (RyR2) have been implicated in some human families with inherited forms of exercise-induced sudden cardiac death. Murine models of calstabin2 deficiency exhibit exercise-induced ventricular arrhythmias and sudden cardiac death. Restoration of calstabin2 binding to RyR2 with small molecules in the 1,4-benzothiazepine class prevent these arrhythmias and sudden death. Several large animals are afflicted by sudden cardiac death including Boxer dogs and cardiomyopathic cats. In cardiomyopathic Boxer dogs that are susceptible to sudden cardiac death, myocardial calstabin2 message and protein were significantly decreased as compared to healthy controls (calstabin2 units per RyR2 complex: affected, 0.51 ± 0.04 ; control, 3.81 ± 0.22 ; $P < 0.0001$). PCR based sequencing of the

promoter, exonic and splice site regions of the canine calstabin gene did not identify any causative mutations. The deficiency of calstabin2 message and protein are a likely cause of ventricular arrhythmias secondary to diastolic SR Ca^{2+} leak in this naturally-occurring large animal model of catecholaminergic polymorphic ventricular tachycardia.

[00174] Exercise-induced sudden cardiac death due to ventricular arrhythmias has been linked to mutations in the cardiac ryanodine receptor (RyR2)/calcium release channel in patients with catecholaminergic polymorphic ventricular tachycardia (CPVT). The RyR2 macromolecular complex controls Ca^{2+} release from the sarcoplasmic reticulum (SR) that is required for cardiac excitation-contraction coupling. RyR2 function is modulated by numerous regulatory molecules in the macromolecular complex including a PKA/PDE/phosphatase module that regulates the PKA phosphorylation of RyR2-Ser2808, which in turn influences the binding affinity of the stabilizing subunit calstabin2 to the RyR2 channel. (Marx,SO, et al., PKA phosphorylation dissociates FKBP12.6 from the calcium release channel (ryanodine receptor): defective regulation in failing hearts. *Cell* 2000;101:365-376). Calstabin2 stabilizes the RyR2 closed state and helps prevent a diastolic SR Ca^{2+} leak that can trigger fatal cardiac arrhythmias and promote cardiac dysfunction following myocardial infarction. (Yano,M, et al. Altered stoichiometry of FKBP12.6 versus ryanodine receptor as a cause of abnormal Ca^{2+} leak through ryanodine receptor in heart failure. *Circulation* 2000;102:2131-2136). Mutations affecting the calstabin2 binding affinity to RyR2 have been reported in CPVT. (Lehnart,SE, et al., Sudden death in familial polymorphic ventricular tachycardia associated with calcium release channel (ryanodine receptor) leak. *Circulation* 2004;109:3208-3214; Kontula,K, Laitinen,PJ, Lehtonen,A, Toivonen,L, Viitasalo,M, Swan,H. Catecholaminergic polymorphic ventricular tachycardia: recent mechanistic insights. *Cardiovasc Res* 2005;67:379-387). Depletion of calstabin2 in the RyR2 complex has also caused been shown to occur during heart failure due to chronic PKA hyperphosphorylation of RyR2. Depletion of calstabin2 from the RyR2 complex causes an increase in the sensitivity of the RyR2 channel to Ca^{2+} -dependent activation such that the calstabin2-depleted RyR2 channels can be activated by very low $[\text{Ca}^{2+}]$, as occurs during cardiac diastole. This pathologic condition results in diastolic SR Ca^{2+} leak, delayed afterdepolarizations, and catecholaminergic ventricular tachycardia. (Lehnart,SE, et al., Stabilization of cardiac ryanodine receptor prevents intracellular calcium leak and arrhythmias. *Proc Natl Acad Sci U.S.A.* 2006;103:7906-7910).

[00175] Boxer dogs exhibit a naturally-occurring form of cardiomyopathy referred to as arrhythmogenic right ventricular cardiomyopathy (ARVC) that has clinical and pathologic similarities to ARVC type 2 and CPVT in humans. (Basso et al, Circulation 2004;109:1180-1185). In particular, affected dogs have a high incidence of fatal ventricular arrhythmias with left bundle branch block morphology, fatty and fibrofatty replacement of right ventricular and occasionally interventricular septum and left ventricular free wall myocardium, and an autosomal dominant mode of inheritance. Thus affected Boxer dogs likely represent a large animal model of calstabin2 deficiency associated with ventricular arrhythmias.

[00176] In one aspect, the present invention provides a model of canine ARVC. In one aspect, the level of calstabin2 (FKBP12.6) is reduced in cardiac tissue of a canine with ARVC. In one embodiment, Boxers with ARVC show alterations in the RyR2-calstabin2 complex. Left ventricular tissues from 4 Boxers with ARVC were obtained at post-mortem. Levels of calstabin2, protein or mRNA in cardiac tissue can be determined by any suitable method known in the art. In one embodiment, mRNA levels of RyR2 and calstabin2 genes were determined using a canine oligonucleotide microarray. In another embodiment, mRNA levels of calstabin2 (FKBP12.6) were determined via real-time RT-PCR. Results demonstrated that calstabin2 (FKBP12.6) mRNA in Boxers was significantly downregulated as compared to healthy control canines and Doberman pinschers with dilated cardiomyopathy (Boxer vs. control, 13.3-fold downregulation; Boxer vs. Doberman, 7.0-fold downregulation). In another embodiment, calstabin2 protein levels are reduced in cardiac tissue of Boxers with ARVC. In one embodiment, analysis by immunoprecipitation and immunoelectrophoresis indicated markedly reduced amounts of calstabin2 in the RyR2-calstabin2 complex of affected Boxers as compared to calstabin2 in the RyR2-calstabin2 complex of controls, while mRNA levels of RyR2 in affected Boxers were unchanged compared to mRNA levels of RyR2 in controls. Therefore, Boxers with ARVC are characterized with calstabin2 deficiency in cardiac tissue. Decrease or loss of calstabin2 may be a cause of the clinical signs associated with ARVC in the Boxer dogs.

[00177] In another aspect, the invention provides a method for determining whether a subject non-human animal has predisposition to an arrhythmogenic condition. In one embodiment, the subject is a non-human animal. In another embodiment, the non-human animal can be, for example but not limited to, selected from the group of canines, felines,

equines, porcine, poultry, ruminants, and rodents. In another embodiment, the arrhythmogenic condition can be selected from, but is not limited to, any one of the conditions from the group of: sudden cardiac death, cardiac arrhythmias, e.g., ventricular and atrial tachycardia; atrial arrhythmia, including atrial tachyarrhythmia and atrial fibrillation (both sustained and non-sustained); ventricular arrhythmia, including ventricular tachyarrhythmia, ventricular fibrillation; and stress or exercise-induced cardiac arrhythmia, catecholaminergic polymorphic ventricular tachycardia (CPVT), arrhythmogenic right ventricular cardiomyopathy (ARVD/C), hypertrophic cardiomyopathy, dilated cardiomyopathy, restrictive cardiomyopathy, sick sinus syndrome, atrial standstill sinus tachycardia, and/or stress- or exercise-induced sudden cardiac death. In one embodiment, the arrhythmogenic condition is ARVC.

[00178] In one aspect, predisposition to an arrhythmogenic condition can be diagnosed based on the levels of calstabin2 in cardiac tissue of a subject animal. A non-limiting example of an arrhythmogenic condition that can be diagnosed by the method of the invention is arrhythmogenic right ventricular cardiomyopathy (ARVC). Determining the levels of mRNA can be done by any method known to a skilled artisan. In one aspect, the levels of calstabin2 in cardiac tissue of a subject non-human animal are determined by any suitable method known in the art. In another aspect, the levels of calstabin2 mRNA in cardiac tissue of a subject non-human animal are determined by any suitable method known in the art. In some embodiments, the levels of calstabin2 mRNA in cardiac tissue are determined by real time reverse transcriptase (RT) PCR assay. In other embodiments, the levels of calstabin2 mRNA in cardiac tissue are determined by conventional RT-PCR. In another embodiment, the levels of calstabin2 mRNA in cardiac tissue are determined by competitive RT-PCR. In another embodiment, the levels of calstabin2 mRNA in cardiac tissue are determined by relative RT-PCR. In another embodiment, the levels of calstabin2 mRNA in cardiac tissue are determined by comparative RT-PCR. In yet another embodiment, the levels of calstabin2 mRNA in cardiac tissue are determined by RNase protection assay as practiced in the art. In another embodiment, the levels of calstabin2 are measured by Northern blot analysis.

[00179] Real time RT-PCR is a sensitive way to measure mRNA levels in samples. Real time RT-PCR enables real time detection of amplification products in samples that are compared. Real time PCR monitors the fluorescence emitted during the reaction as an

indicator of product synthesis during the reaction. Quantitation by real time PCR does not require post PCR processing of the PCR products, which allows increased throughput and reduced the probability of carryover contamination. Quantitative real time RT-PCR can be done as one step real-time RT-PCR or two step real-time RT-PCR. One-step real-time RT-PCR performs reverse transcription and PCR in a single buffer system and in one tube. In two-step real time RT-PCR, these two steps are performed separately in different tubes. Methods on how to perform one and two step real-time PCR are well known in the art.

[00180] There are several different chemistries that can be used to monitor fluorescence emission in real time PCR amplification reaction. TaqMan® (Applied Biosystems, Foster City, CA, USA), Molecular Beacons, Scorpions® and SYBR® Green (Molecular Probes), are available for real-time PCR. TaqMan® probes are generally longer than primers (about 15-30 bases long) and contain a fluorescent dye usually at the 5' end and a quenching dye (for example 6-carboxy-terta-methyl-rhodamine TAMRA) at the at the 3' end. Molecular beacons also contain fluorescent (for example but not limited to, 6-carboxy fluorescein (FAM), TAMRA, TET, 6-carboxy-X-rhodamine (ROX)) and quenching dyes (for example DABCYL) at either end but these probes are designed to adopt a hairpin structure while free in solution so that the fluorescent dye and quencher are brought in close proximity for FRET to occur. When the beacon hybridises to the target during the annealing step, the reporter dye is separated from the quencher and the reporter fluoresces. Scorpion probes allow sequence-specific priming and PCR product detection using a single oligonucleotide. The Scorpion probe maintains a stem-loop configuration in the unhybridised state. The fluorophore is attached to the 5' end and is quenched by a moiety coupled to the 3' end. The 3' portion of the stem also contains a sequence that is complementary to the extension product of the primer. This sequence is linked to the 5' end of a specific primer via a non-amplifiable monomer. After extension of the Scorpion primer, the specific probe sequence is able to bind to its complement within the extended amplicon thus opening up the hairpin loop. This prevents the fluorescence from being quenched and a signal is observed.

[00181] All of the above-described chemistries allow detection of PCR products via the generation of a fluorescent signal. TaqMan probes, Molecular Beacons and Scorpions depend on Förster Resonance Energy Transfer (FRET) to generate the fluorescence signal via the coupling of a fluorogenic dye molecule and a quencher moiety to the same or

different oligonucleotide substrates. SYBR Green is a fluorogenic dye that exhibits little fluorescence when in solution, but emits a strong fluorescent signal upon binding to double-stranded DNA. Although real time PCR does not detect the size of the PCR product it is generally not influenced by non-specific amplification, unless a double stranded (ds) DNA binding dye is used. When DNA binding dye is used, melting curve analysis of the product in a real time PCR reaction can be performed to determine whether the product is specific. All real-time PCR chemistries allow multiplexing, detection of multiple DNA species, by designing each probe/beacon with a spectrally unique fluor/quench pair as long as the platform is suitable for melting curve analysis if SYBR green is used. By multiplexing, the target(s) and endogenous control can be amplified in single tube.

[00182] Based on the fluorescence intensity recorded during the real time amplification, there are several ways to quantitate the amount of template. For example, several strategies are commonly employed to quantify the results obtained by real-time PCR, including real time real time RT-PCR: the standard curve method and the comparative threshold method. In the standard curve method, a standard curve is first constructed from RNA of known concentration. This curve is then used as a reference standard for extrapolating quantitative information for mRNA targets of unknown concentrations. Though RNA standards can be used, their stability can be a source of variability in the final analyses. The use of absolutely quantitated RNA standards will allow generation of absolute copy number data. Alternatively, other nucleic acid samples can be used to construct the standard curve, including purified plasmid dsDNA, in vitro generated ssDNA or any cDNA sample expressing the target gene. Concentration measurements of these DNAs can be converted to a copy number value based on the molecular weight of the sample used. cDNA plasmids are the preferred standards for standard curve quantitation. This method will yield information on relative changes in mRNA expression because cDNA plasmids do not account for variations in the efficiency of the reverse transcription step. This, and variation introduced due to variable RNA inputs, can be corrected by normalization to a housekeeping gene.

[00183] Another method for real time RT-PCR quantitation is the C_t method. The threshold cycle C_t parameter is defined as the cycle number at which fluorescence emission exceeds a fixed threshold level. The threshold cycle is when the system begins to detect the increase in the signal associated with exponential growth of PCR product during the log-

linear phase. The comparative C_t method involves comparing the (threshold cycle) C_t values of the samples of interest with a control or calibrator such as a non-treated sample or RNA from normal tissue. The C_t values of both the calibrator and the samples of interest are normalized to an appropriate endogenous housekeeping gene. If the plot of cDNA dilution versus ΔC_t is close to zero, it implies that the efficiencies of the target and housekeeping genes are very similar. If a housekeeping gene cannot be found whose amplification efficiency is similar to the target, then the standard curve method can be used. Another method for analysis of amplification is the comparative C_t method for relative quantitation of gene expression. In this method the calculation involves finding the difference between each sample's ΔC_t and the baseline 's ΔC_t .

[00184] Relative, competitive and comparative end-point RT-PCR can be used to measure changes in mRNA expression levels. Methods for quantitating end-point RT-PCR results rely on detecting a fluorescent dye such as ethidium bromide, quantitation of P^{32} -labeled PCR product by a phosphorimager, by scintillation counting other methods known in the art. Relative RT-PCR quantitation compares transcript levels across multiple samples, using a co-amplified internal control to normalize between samples. Results can be expressed as ratios of the gene-specific signal to the internal control signal. This yields a corrected relative value for the gene-specific product in each sample. These values may be compared between samples for an estimate of the relative expression of target RNA in the samples thus providing fold difference of expression levels between difference samples. To measure absolute amount of mRNA, the PCR product from the endogenous transcript can be compared to a concentration curve created by a synthetic competitor RNA. Dilutions of a competitor RNA, synthetic RNA of identical but shorter sequence, is added to sample RNA replicates and are co-amplified with the endogenous target. Comparative RT-PCR is similar to competitive RT-PCR in that target message from each RNA sample competes for amplification reagents within a single reaction, making the technique reliably quantitative. The Comparative RT-PCR method relies on reverse transcription of two RNA samples. Each RNA sample is reverse transcribed to cDNA with a different anchored oligo(dT) primer, wherein the 5' ends of these primers have identical PCR primer binding sites, but the nucleotide sequences linking the primer binding site to the oligo(dT) are different lengths. Because the cDNA from both samples have the same PCR primer-binding site, one sample acts as a competitor for the other, making it unnecessary to synthesize a competitor RNA sequence.

[00185] In another aspect, the invention provides synthetic nucleic acids comprising from about 10 to about 55 consecutive nucleotides, from about 10 to about 35 consecutive nucleotides, from about 10 to about 30 consecutive nucleotides, from about 10 to about 25, from about 10 to about 20 consecutive nucleotides, from about 10 to about 15 consecutive nucleotides from SEQ ID NO: 1. SEQ ID NO: 1 illustrates the nucleic acid sequence of canine calstabin2 as shown in FIG. 2 embodiment A.

[00186] In another aspect, the invention provides synthetic nucleic acids comprising from about 10 to about 55 consecutive nucleotides, from about 10 to about 35 consecutive nucleotides, from about 10 to about 30 consecutive nucleotides, from about 10 to about 25, from about 10 to about 20 consecutive nucleotides, from about 10 to about 15 consecutive nucleotides from a sequence which is complementary to SEQ ID NO: 1.

[00187] In another aspect any one of the synthetic nucleic acids can further comprise at least one fluorescent label. Non-limiting examples of fluorescent labels are: 6-carboxy fluorescein (FAM), TAMRA, TET, 6-carboxy-X-rhodamine (ROX). In an embodiment wherein the synthetic nucleic acid comprises two fluorescent labels, the two labels are different from each other. Synthetic nucleic acids marked with different fluorescent labels can be used in real time PCR assays, for example but not limited to real time RT-PCR.

[00188] The invention further provides a composition comprising one or more of the synthetic nucleic acid sequences comprising from about 10 to about 35 consecutive nucleotides from SEQ ID NO: 1. The invention further provides a composition comprising one or more of the synthetic nucleic acid sequences comprising from about 10 to about 35 consecutive nucleotides from a sequence which is complementary to SEQ ID NO: 1. The invention further provides a composition comprising one or more of the synthetic nucleic acid sequences comprising from about 10 to about 35 consecutive nucleotides from SEQ ID NO: 1, or a sequence which is complementary to SEQ ID NO: 1.

[00189] In one embodiment, the synthetic nucleic acid which comprises about 10 to about 35 consecutive nucleotides from SEQ ID NO: 1, or from about 10 to about 35 consecutive nucleotides from a sequence which is complementary to SEQ ID NO: 1, is a primer. Primers of the invention can be used in PCR assays, for example but not limited to real time RT-PCR assay, to determine whether a canine subject has differential level of calstabin2 mRNA expression. The invention further provides a primer set comprising at

least two synthetic nucleic acid sequences. A primer set of the invention can comprise at least one of the synthetic nucleic acids comprising from about 10 to about 35 consecutive nucleotides from SEQ ID NO: 1, and/or at least one of the synthetic nucleic acids comprising from about 10 to about 35 consecutive nucleotides from a sequence which is complementary to SEQ ID NO: 1.

[00190] The invention further provides a kit for determining the levels of calstabin2 mRNA in a sample, comprising at least one of the synthetic nucleic acids comprising from about 10 to about 35 consecutive nucleotides from SEQ ID NO: 1, or from about 10 to about 35 consecutive nucleotides from a sequence which is complementary to SEQ ID NO: 1. The kit further comprising optional PCR reagents and positive/negative control for determining the levels of calstabin2 mRNA in a sample.

[00191] In another aspect, the levels of calstabin2 mRNA in cardiac tissue can be measured by a method that uses an oligonucleotide array comprising canine nucleic acid sequences. Canine microarrays can be custom made. Alternatively, the Affymetrix GeneChip® Canine Genome Array, or any other commercially available canine microarray, can be used. Samples comprising mRNAs isolated from normal dogs or candidate subject dogs suspected to have a cardiac condition are used in a hybridization experiment with a canine oligonucleotide microarrays to determine whether a gene of interest, for example Calstabin2, is differentially expressed in the normal versus candidate subject dog. RNA samples for use in real time RT-PCR or microarray hybridization experiments can be prepared by any suitable method known in the art. When necessary, total mRNA is reverse transcribed into cDNA by any suitable method known in the art.

[00192] In another aspect, the levels of calstabin2 protein in a cardiac tissue, including but not limited to the level of calstabin2 in the RyR2-calstabin2 complex in a cardiac tissue, of a subject non-human animal are determined by any suitable method for protein analysis known in the art. Non-limiting examples of such methods are immunological techniques, hybridization analysis, immunoprecipitation, Western-blot analysis, fluorescence imaging techniques and/or radiation detection, as well as any assays and detection methods disclosed herein. For example, protein is isolated and purified from cells of a subject using standard methods known in the art, including, without limitation, extraction from the cells (*e.g.*, with a detergent that solubilizes the protein) where necessary, followed by affinity purification on a column, chromatography (*e.g.*, FTLC and HPLC),

immunoprecipitation (with an antibody), and precipitation (*e.g.*, with isopropanol and a reagent such as Trizol). Isolation and purification of the protein is followed by electrophoresis (*e.g.*, on an SDS-polyacrylamide gel).

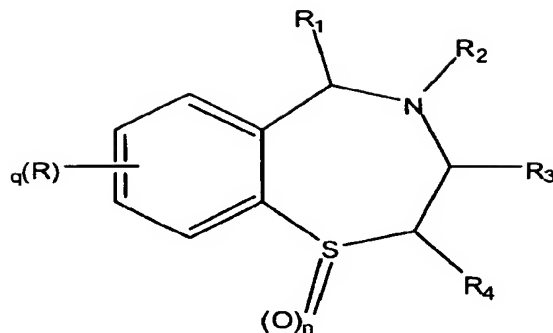
[00193] Determining predisposition to an arrhythmogenic condition based on the level of calstabin2 in cardiac tissue can optionally include any other method, for example but not limited to methods such a short 2-3 minute ECG, an ambulatory 24-hour ECG (Holter monitor), echocardiography and Doppler echocardiography.

[00194] The foregoing discussion illustrates that leaks in RyR2 channels are associated with a number of pathological states – in both diseased hearts and structurally normal hearts. Accordingly, methods and composition are necessary to repair the Ca²⁺ leaks in RyR2 complex, and/or restore the ratio of calstabin2 to RyR2 in the RyR2-calstabin complex. Such methods and composition could prevent heart failure, and fatal arrhythmias and fibrillations, in millions of patients and non-human animals, including companion animals, including but not limited to canines, felines, and rodents.

Compounds

[00195] JTV-519 (4-[3-(4-benzylpiperidin-1-yl)propionyl]-7-methoxy-2,3,4,5-tetrahydro-1,4-benzothiazepine monohydrochloride; also known as k201 or ICP-Calstan 100), a derivative of 1,4-benzothiazepine, is a new modulator of calcium-ion channels. In addition to regulating Ca²⁺ levels in myocardial cells, JTV-519 also modulates the Na⁺ current and the inward-rectifier K⁺ current in guinea pig ventricular cells, and inhibits the delayed-rectifier K⁺ current in guinea pig atrial cells. Studies have shown that JTV-519 has a strong cardioprotective effect against catecholamine-induced myocardial injury, myocardial-injury-induced myofibrillar overcontraction, and ischemia/reperfusion injury. In experimental myofibrillar overcontraction models, JTV-519 demonstrated greater cardioprotective effects than propranolol, verapamil, and diltiazem. Experimental data also suggest that JTV-519 effectively prevents ventricular ischemia/reperfusion by reducing the level of intracellular Ca²⁺ overload in animal models.

[00196] The present invention further provides veterinary uses of compounds of Formula I, I-a, I-b, I-c, I-d, I-e, I-f, I-g, I-h, I-i, I-j, I-k, I-l, I-m or Formula II. Compounds of Formula I have the following structure:



wherein,

n is 0, 1, or 2;

q is 0, 1, 2, 3, or 4;

each R is independently selected from the group consisting of H, halogen, -OH, -NH₂, -NO₂, -CN, -CF₃, -OCF₃, -N₃, -SO₃H, -S(=O)₂alkyl, -S(=O)alkyl, -OS(=O)₂CF₃, acyl, -O-acyl, alkyl, alkoxyl, alkylamino, alkylarylamino, alkylthio, cycloalkyl, alkylaryl, aryl, heteroaryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino; wherein each acyl, -O-acyl, alkyl, alkoxyl, alkylamino, alkylarylamino, alkylthio, cycloalkyl, alkylaryl, aryl, heteroaryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino may be optionally substituted;

R₁ is selected from the group consisting of H, oxo, alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl; wherein each alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl may be optionally substituted;

R₂ is selected from the group consisting of H, -C(=O)R₅, -C(=S)R₆, -SO₂R₇, -P(=O)R₈R₉, -(CH₂)_m-R₁₀, alkyl, aryl, alkylaryl, heteroaryl, cycloalkyl, cycloalkylalkyl, and heterocyclyl; wherein each alkyl, aryl, alkylaryl, heteroaryl, cycloalkyl, cycloalkylalkyl, and heterocyclyl may be optionally substituted;

R₃ is selected from the group consisting of H, -CO₂Y, -C(=O)NHY, acyl, -O-acyl, alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl; wherein each acyl, alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl may be optionally substituted; and wherein Y is selected from the group consisting of H, alkyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl, and wherein each alkyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl may be optionally substituted;

R₄ is selected from the group consisting of H, alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl; wherein each alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl may be optionally substituted;

R₅ is selected from the group consisting of -NR₁₅R₁₆, -(CH₂)_qNR₁₅R₁₆, -NHNHNR₁₅R₁₆, -NHOH, -OR₁₅, -C(=O)NHNHNR₁₅R₁₆, -CO₂R₁₅, -C(=O)NR₁₅R₁₆, -CH₂X, acyl, alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl may be optionally substituted, and wherein q is 1, 2, 3, 4, 5, or 6;

R₆ is selected from the group consisting of -OR₁₅, -NHNHNR₁₅R₁₆, -NHOH, -NR₁₅R₁₆, -CH₂X, acyl, alkenyl, alkyl, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkenyl, alkyl, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl may be optionally substituted;

R₇ is selected from the group consisting of -OR₁₅, -NR₁₅R₁₆, -NHNHNR₁₅R₁₆, -NHOH, -CH₂X, alkyl, alkenyl, alkynyl, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl; wherein each alkyl, alkenyl, alkynyl, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl may be optionally substituted;

R₈ and R₉ independently are selected from the group consisting of OH, acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl may be optionally substituted;

R₁₀ is selected from the group consisting of -NR₁₅R₁₆, OH, -SO₂R₁₁, -NHSO₂R₁₁, C(=O)(R₁₂), NHC=O(R₁₂), -OC=O(R₁₂), and -P(=O)R₁₃R₁₄;

R₁₁, R₁₂, R₁₃, and R₁₄ independently are selected from the group consisting of H, OH, NH₂, -NHNH₂, -NHOH, acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl may be optionally substituted;

X is selected from the group consisting of halogen, -CN, -CO₂R₁₅, -C(=O)NR₁₅R₁₆, -NR₁₅R₁₆, -OR₁₅, -SO₂R₇, and -P(=O)R₈R₉; and

R₁₅ and R₁₆ independently are selected from the group consisting of H, acyl, alkenyl, alkoxyl, OH, NH₂, alkyl, alkylamino, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclalkyl; wherein each acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclalkyl may be optionally substituted; and optionally R₁₅ and R₁₆ together with the N to which they are bonded may form a heterocycle which may be substituted;

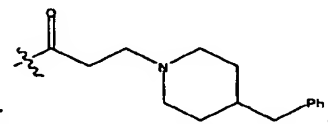
the nitrogen in the benzothiazepine ring may optionally be a quaternary nitrogen; and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, and prodrugs thereof;

provided that when q is 0 and n is 0, then R₂ is not H, Et, -C(=O)NH₂, (=O)NHPh, -C(=S)NH-nButyl, -C(=O)NHC(=O)CH₂Cl, -C(=O)H, -C(=O)Me, -C(=O)Et, -C(=O)CH=CH₂, -S(=O)₂Me, or -S(=O)₂Et;

further provided that when q is 0 and n is 1 or 2, then R₂ is not -C(=O)Me, -C(=O)Et, -S(=O)₂Me, or -S(=O)₂Et;

further provided that when q is 1, and R is Me, Cl, or F at the 6 position of the benzothiazepene ring, then R₂ is not H, Me, -C(=O)H, -C(=O)Me, -C(=O)Et, -C(=O)Ph, -S(=O)₂Me, or -S(=O)₂Et; and

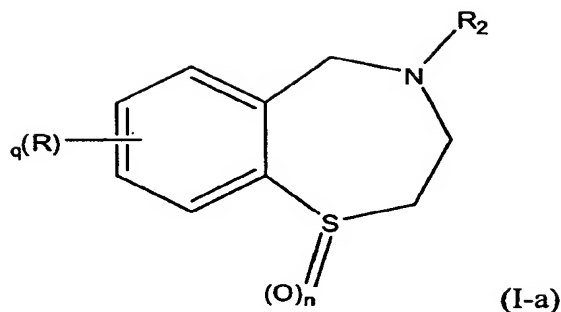
further provided that when q is 1, n is 0, and R is OCT₃, OH, C₁-C₃ alkoxyl at the 7 position



of the benzothiazepene ring, then R₂ is not H, -C(=O)CH=CH₂, or

[00197] In one embodiment, the present invention provides compounds of Formula I, as described above, with the proviso that the compound is not S24 or S68.

[00198] In one embodiment, the present invention provides compounds of Formula I-a:



wherein:

n is 0, 1, or 2;

q is 0, 1, 2, 3, or 4;

each R is independently selected from the group consisting of H, halogen, -OH, -NH₂, -NO₂, -CN, -CF₃, -OCF₃, -N₃, -SO₃H, -S(=O)₂alkyl, -S(=O)alkyl, -OS(=O)₂CF₃, acyl, alkyl, alkoxy, alkylamino, alkylthio, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino; wherein each acyl, alkyl, alkoxy, alkylamino, alkylthio, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino may be substituted or unsubstituted;

R_2 is selected from the group consisting of H, -C(=O)(R₅), -C(=S)(R₆), -SO₂R₇, -P(=O)R₈R₉, -(CH₂)_m-R₁₀, alkyl, aryl, heteroaryl, cycloalkyl, cycloalkylalkyl, and heterocyclyl; wherein each alkyl, aryl, heteroaryl, cycloalkyl, cycloalkylalkyl, and heterocyclyl may be substituted or unsubstituted;

R_5 is selected from the group consisting of -NR₁₅R₁₆, -NHNHNR₁₅R₁₆, -NHOH, -OR₁₅, -C(=O)NHNHNR₁₅R₁₆, -CO₂R₁₅, -C(=O)NR₁₅R₁₆, -CH₂X, acyl, alkyl, alkenyl, alkynyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkyl, alkenyl, alkynyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted;

R_6 is selected from the group consisting of -OR₁₅, -NHNHNR₁₅R₁₆, -NHOH, -NR₁₅R₁₆, -CH₂X, acyl, alkenyl, alkyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkenyl, alkyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted;

R₇ is selected from the group consisting of H, -OR₁₅, -NR₁₅R₁₆, -NHNHNR₁₅R₁₆, -NHOH, -CH₂X, alkyl, akenyl, alkynyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each alkyl, akenyl, alkynyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted;

R₈ and R₉ independently are selected from the group consisting of -OH, acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted;

R₁₀ is selected from the group consisting of -NR₁₅R₁₆, OH, -SO₂R₁₁, -NHCO₂R₁₁, -C(=O)R₁₂, -NH(C=O)R₁₂, -O(C=O)R₁₂, and -P(=O)R₁₃R₁₄;

m is 0, 1, 2, 3, or 4;

R₁₁, R₁₂, R₁₃, and R₁₄ independently are selected from the group consisting of H, OH, NH₂, -NHNH₂, -NHOH, acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted;

X is selected from the group consisting of halogen, -CN, -CO₂R₁₅, -C(=O)NR₁₅R₁₆, -NR₁₅R₁₆, -OR₁₅, -SO₂R₇, and -P(=O)R₈R₉; and

R₁₅ and R₁₆ independently are selected from the group consisting of H, acyl, alkenyl, alkoxyl, OH, NH₂, alkyl, alkylamino, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted; and optionally R₁₅ and R₁₆ together with the N to which they are bonded may form a heterocycle which may be substituted or unsubstituted;

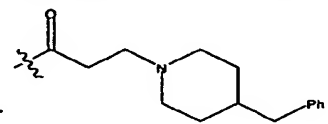
the nitrogen in the benzothiazepine ring may be optionally a quaternary nitrogen; and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, and prodrugs thereof;

provided that when q is 0 and n is 0, then R₂ is not H, Et, -C(=O)NH₂, (=O)NHPh, -C(=S)NH-nButyl, -C(=O)NHC(=O)CH₂Cl, -C(=O)H, -C(=O)Me, -C(=O)Et, -C(=O)CH=CH₂, -S(=O)₂Me, or -S(=O)₂Et;

further provided that when q is 0 and n is 1 or 2, then R_2 is not $-C(=O)Me$, $-C(=O)Et$, $-S(=O)_2Me$, or $-S(=O)_2Et$;

further provided that when q is 1, and R is Me, Cl, or F at the 6 position of the benzothiazepene ring, then R_2 is not H, Me, $-C(=O)H$, $-C(=O)Me$, $-C(=O)Et$, $-C(=O)Ph$, $-S(=O)_2Me$, or $-S(=O)_2Et$; and

further provided that when q is 1, n is 0, and R is OCT_3 , OH, C_1 - C_3 alkoxy at the 7 position

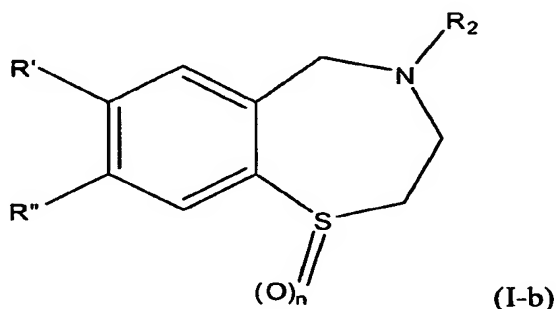


of the benzothiazepene ring, then R_2 is not H, $-C(=O)CH=CH_2$, or

[00199] In certain embodiments, the present invention provides compounds of formula **I-a**, wherein each R is independently selected from the group consisting of H, halogen, $-OH$, OMe , $-NH_2$, $-NO_2$, $-CN$, $-CF_3$, $-OCF_3$, $-N_3$, $-S(=O)_2C_1$ - C_4 alkyl, $-S(=O)C_1$ - C_4 alkyl, $-S-C_1$ - C_4 alkyl, $-OS(=O)_2CF_3$, Ph, $-NHCH_2Ph$, $-C(=O)Me$, $-OC(=O)Me$, morpholinyl and propenyl; and n is 0, 1, or 2.

[00200] In other embodiments, the present invention provides compounds of formula **I-a**, wherein R_2 is selected from the group consisting of $-C=O(R_5)$, $-C=S(R_6)$, $-SO_2R_7$, $-P(=O)R_8R_9$, and $-(CH_2)_m-R_{10}$.

[00201] In yet another embodiment, the present invention provides compounds of formula **I-b**:



wherein R' and R'' are independently selected from the group consisting of H, halogen, $-OH$, $-NH_2$, $-NO_2$, $-CN$, $-CF_3$, $-OCF_3$, $-N_3$, $-SO_3H$, $-S(=O)_2$ alkyl, $-S(=O)$ alkyl, $-OS(=O)_2CF_3$, acyl, alkyl, alkoxy, alkylamino, alkylthio, cycloalkyl, aryl, heterocyclyl, heterocyclalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)aryl amino; and wherein each

acyl, alkyl, alkoxyl, alkylamino, cycloalkyl, aryl, heterocyclyl, heterocyclalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio may be substituted or unsubstituted;

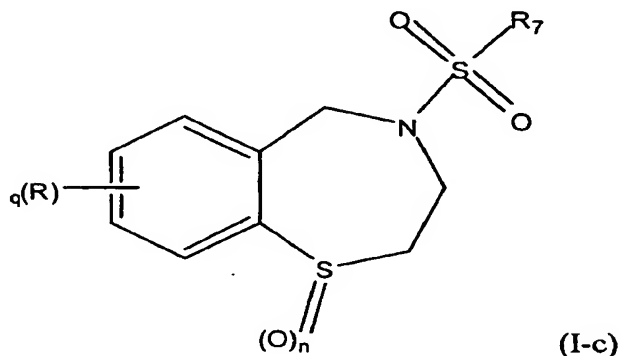
R_2 and n are as defined in compounds of formula **I-a** above;

and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

[00202] In certain embodiments, the present invention provides compounds of formula **I-b**, wherein R' and R'' are independently selected from the group consisting of H, halogen, -OH, OMe, -NH₂, -NO₂, -CN, -CF₃, -OCF₃, -N₃, -S(=O)₂C₁-C₄alkyl, -S(=O)C₁-C₄alkyl, -S-C₁-C₄alkyl, -OS(=O)₂CF₃, Ph, -NHCH₂Ph, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1 or 3. In some cases, R' is H or OMe, and R'' is H.

[00203] In other embodiments, the present invention provides compounds of formula **I-b**, wherein R_2 is selected from the group consisting of -C=O(R_5), -C=S(R_6), -SO₂ R_7 , -P(=O) R_8R_9 , and -(CH₂) _{m} - R_{10} .

[00204] In yet another embodiment, the present invention provides compounds formula of **I-c**:



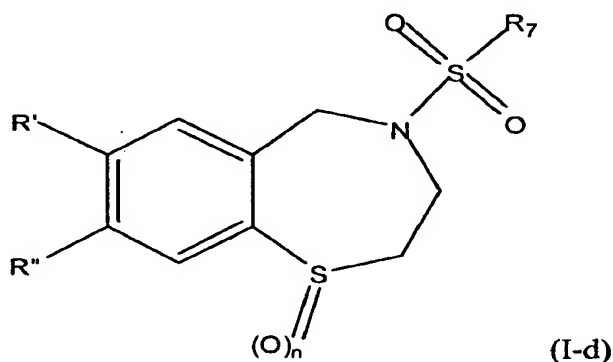
wherein each R , R_7 , q , and n is as defined in compounds of formula **I-a** above; and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

[00205] In certain embodiments, the present invention provides compounds of formula **I-c**, wherein each R is independently selected from the group consisting of H, halogen, -OH, OMe, -NH₂, -NO₂, -CN, -CF₃, -OCF₃, -N₃, -S(=O)₂C₁-C₄alkyl, -S(=O)C₁-

C₄alkyl, -S-C₁-C₄alkyl, -OS(=O)₂CF₃, Ph, -NHCH₂Ph, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1, or 2.

[00206] In other embodiments, the present invention provides compounds of formula **I-c**, wherein R₇ is selected from the group consisting of -OH, -NR₁₅R₁₆, alkyl, alkenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each alkyl, alkenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted.

[00207] In a further embodiment, the present invention provides compounds of formula of **I-d**:



wherein R' and R'' are independently selected from the group consisting of H, halogen, -OH, -NH₂, -NO₂, -CN, -CF₃, -OCF₃, -N₃, -SO₃H, -S(=O)₂alkyl, -S(=O)alkyl, -OS(=O)₂CF₃, acyl, alkyl, alkoxy, alkylamino, alkylthio, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)aryl amino; and wherein each acyl, alkyl, alkoxy, alkylamino, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio may be substituted or unsubstituted;

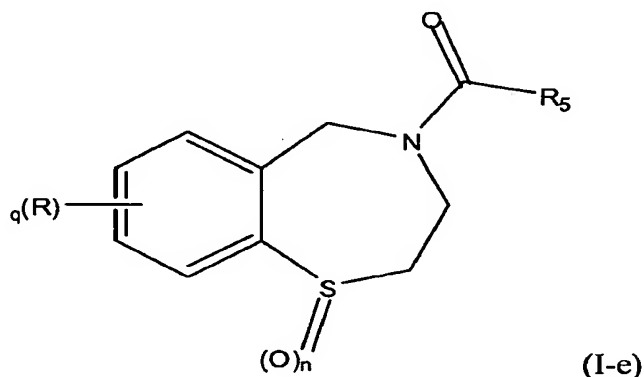
R₇ and n are as defined in compounds of formula **I-a** above; and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

[00208] In certain embodiments, the present invention provides compounds of formula **I-d**, wherein R' and R'' are independently selected from the group consisting of H, halogen, -OH, OMe, -NH₂, -NO₂, -CN, -CF₃, -OCF₃, -N₃, -S(=O)₂C₁-C₄alkyl, -S(=O)C₁-

C₄alkyl, -S-C₁-C₄alkyl, -OS(=O)₂CF₃, Ph, -NHCH₂Ph, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1 or 3. In some cases, R' is H or OMe, and R'' is H.

[00209] In other embodiments, the present invention provides compounds of formula **I-d**, wherein R₇ is selected from the group consisting of -OH, -NR₁₅R₁₆, alkyl, alkenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclalkyl; wherein each alkyl, alkenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclalkyl may be substituted or unsubstituted.

[00210] In one embodiment, the present invention provides compounds of formula of **I-e**:



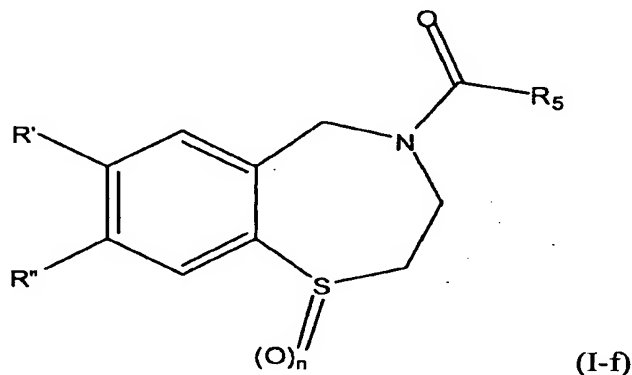
wherein each R, R₅, q and n is as defined compounds of formula **I-a** above; and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

[00211] In certain embodiments, the present invention provides compounds of formula **I-e**, wherein each R is independently selected from the group consisting of H, halogen, -OH, OMe, -NH₂, -NO₂, -CN, -CF₃, -OCF₃, -N₃, -S(=O)₂C₁-C₄alkyl, -S(=O)C₁-C₄alkyl, -S-C₁-C₄alkyl, -OS(=O)₂CF₃, Ph, -NHCH₂Ph, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1, or 2.

[00212] In other embodiments, the present invention provides compounds of formula **I-e**, wherein R₅ is selected from the group consisting of -NR₁₅R₁₆, -NHOH, -OR₁₅, -CH₂X, alkyl, alkenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclalkyl; wherein each acyl, alkyl, alkenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclalkyl may be substituted or unsubstituted.

[00213] In some embodiments, the present invention provides compounds of formula I-e, wherein R_5 is an alkyl substituted by at least one labeling group, such as a fluorescent, a bioluminescent, a chemiluminescent, a colorimetric and a radioactive labeling group. A fluorescent labeling group can be selected from bodipy, dansyl, fluorescein, rhodamine, Texas red, cyanine dyes, pyrene, coumarins, Cascade BlueTM, Pacific Blue, Marina Blue, Oregon Green, 4',6-Diamidino-2-phenylindole (DAPI), indopyra dyes, lucifer yellow, propidium iodide, porphyrins, arginine, and variants and derivatives thereof.

[00214] In another embodiment, the present invention provides compounds of formula of I-f:



wherein R' and R'' are independently selected from the group consisting of H, halogen, -OH, -NH₂, -NO₂, -CN, -CF₃, -OCF₃, -N₃, -SO₃H, -S(=O)₂alkyl, -S(=O)alkyl, -OS(=O)₂CF₃, acyl, alkyl, alkoxyl, alkylamino, alkylthio, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylmino; and wherein each acyl, alkyl, alkoxyl, alkylamino, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio may be substituted or unsubstituted;

R_5 and n are as defined in compounds of formula I-a above;

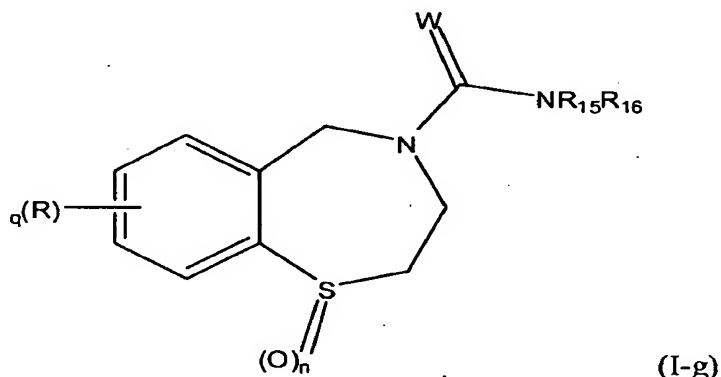
and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

[00215] In certain embodiments, the present invention provides compounds of formula I-f, wherein R' and R'' are independently selected from the group consisting of H, halogen, -OH, OMe, -NH₂, -NO₂, -CN, -CF₃, -OCF₃, -N₃, -S(=O)₂C₁-C₄alkyl, -S(=O)C₁-

C₄alkyl, -S-C₁-C₄alkyl, -OS(=O)₂CF₃, Ph, -NHCH₂Ph, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1 or 3. In some cases, R' is H or OMe, and R'' is H.

[00216] In other embodiments, the present invention provides compounds of formula I-f, wherein R₅ is selected from the group consisting of -NR₁₅R₁₆, -NHOH, -OR₁₅, -CH₂X, alkyl, alkenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkyl, alkenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted.

[00217] In yet another embodiment, the present invention provides compounds of formula of I-g:



wherein W is S or O; each R, R₁₅, R₁₆, q, and n is as defined in compounds of formula I-a above; and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

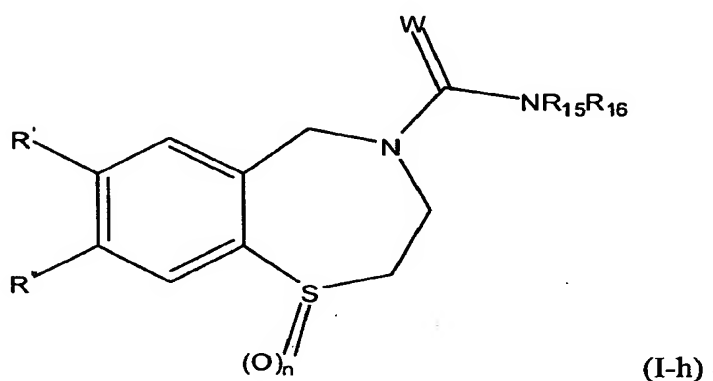
[00218] In certain embodiments, the present invention provides compounds of formula I-g, wherein each R is independently selected from the group consisting of H, halogen, -OH, OMe, -NH₂, -NO₂, -CN, -CF₃, -OCF₃, -N₃, -S(=O)₂C₁-C₄alkyl, -S(=O)C₁-C₄alkyl, -S-C₁-C₄alkyl, -OS(=O)₂CF₃, Ph, -NHCH₂Ph, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1, or 2.

[00219] In other embodiments, the present invention provides compounds of formula I-g, wherein R₁₅ and R₁₆ independently are selected from the group consisting of H, OH, NH₂, alkyl, alkylamino, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each alkyl, alkylamino, aryl, cycloalkyl, cycloalkylalkyl,

heterocyclyl, and heterocyclylalkyl may be substituted; and optionally R_{15} and R_{16} together with the N to which they are bonded may form a heterocycle which may be substituted.

[00220] In some embodiments, the present invention provides compounds of formula **I-g**, wherein W is O or S.

[00221] In yet another embodiment, the present invention provides compounds of formula of **I-h**:



wherein W is S or O;

wherein R' and R'' are independently selected from the group consisting of H, halogen, -OH, -NH₂, -NO₂, -CN, -CF₃, -OCF₃, -N₃, -SO₃H, -S(=O)₂alkyl, -S(=O)alkyl, -OS(=O)₂CF₃, acyl, alkyl, alkoxyl, alkylamino, alkylthio, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)aryl amino; and wherein each acyl, alkyl, alkoxyl, alkylamino, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio may be substituted or unsubstituted;

R_{15} , R_{16} and n are as defined in compounds of formula **I-a** above;

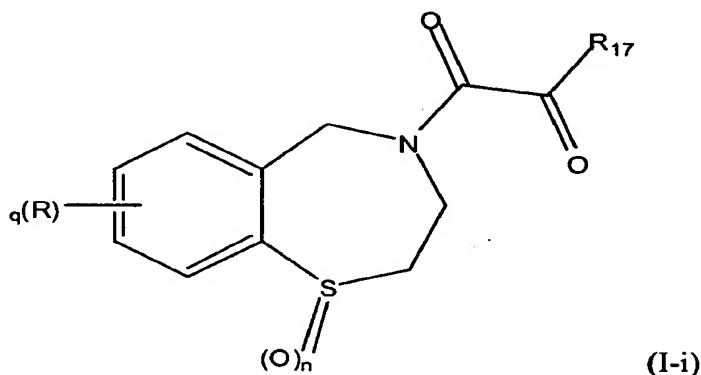
and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

[00222] In certain embodiments, the present invention provides compounds of formula **I-h**, wherein R' and R'' are independently selected from the group consisting of H, halogen, -OH, OMe, -NH₂, -NO₂, -CN, -CF₃, -OCF₃, -N₃, -S(=O)₂C₁-C₄alkyl, -S(=O)C₁-C₄alkyl, -S-C₁-C₄alkyl, -OS(=O)₂CF₃, Ph, -NHCH₂Ph, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1 or 3. In some cases, R' is H or OMe, and R'' is H.

[00223] In other embodiments, the present invention provides compounds of formula I-h, wherein R_{15} and R_{16} independently are selected from the group consisting of H, OH, NH_2 , alkyl, alkylamino, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each alkyl, alkylamino, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted; and optionally R_{15} and R_{16} together with the N to which they are bonded may form a heterocycle which may be substituted.

[00224] In some embodiments, the present invention provides compounds of formula I-g, wherein W is O or S.

[00225] In a further embodiment, the present invention provides compounds of formula of I-i:



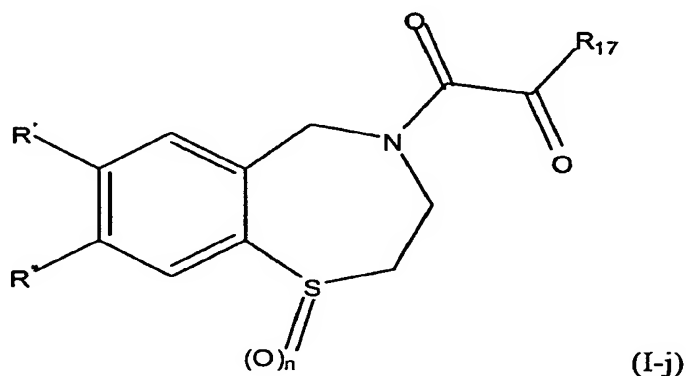
wherein R_{17} is selected from the group consisting of $-NR_{15}R_{16}$, $-NHN R_{15}R_{16}$, $-NHOH$, $-OR_{15}$, $-CH_2X$, alkenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each alkenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted;

each R, q, and n is as defined in compounds of formula I-a above; and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

[00226] In certain embodiments, the present invention provides compounds of formula I-i, wherein each R is independently selected from the group consisting of H, halogen, $-OH$, OMe , $-NH_2$, $-NO_2$, $-CN$, $-CF_3$, $-OCF_3$, $-N_3$, $-S(=O)_2C_1-C_4alkyl$, $-S(=O)C_1-C_4alkyl$, $-S-C_1-C_4alkyl$, $-OS(=O)_2CF_3$, Ph , $-NHCH_2Ph$, $-C(=O)Me$, $-OC(=O)Me$, morpholinyl and propenyl; and n is 0, 1, or 2.

[00227] In other embodiments, the present invention provides compounds of formula I-i, wherein R_{17} is $-NR_{15}R_{16}$, and $-OR_{15}$. In certain other embodiments, R_{17} is $-OH$, $-OMe$, $-NEt$, $-NHet$, $-NHPh$, $-NH_2$, or $-NHCH_2pyridyl$.

[00228] In one embodiment, the present invention provides compounds of formula of I-j:



wherein R' and R'' are independently selected from the group consisting of H, halogen, $-OH$, $-NH_2$, $-NO_2$, $-CN$, $-CF_3$, $-OCF_3$, $-N_3$, $-SO_3H$, $-S(=O)_2alkyl$, $-S(=O)alkyl$, $-OS(=O)_2CF_3$, acyl, alkyl, alkoxyl, alkylamino, alkylthio, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino; and wherein each acyl, alkyl, alkoxyl, alkylamino, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio may be substituted or unsubstituted;

R_{17} is selected from the group consisting of $-NR_{15}R_{16}$, $-NHN R_{15}R_{16}$, $-NHOH$, $-OR_{15}$, $-CH_2X$, alkenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each alkenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted;

n is as defined in compounds of formula I-a; and

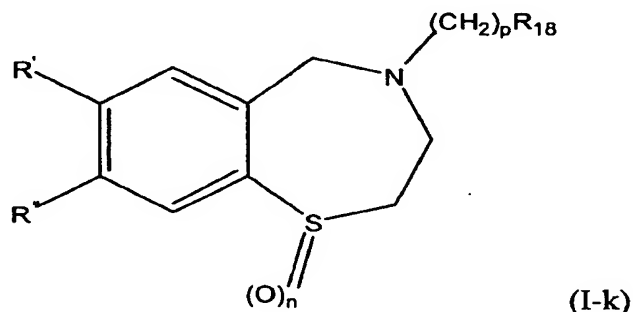
enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

[00229] In certain embodiments, the present invention provides compounds of formula I-j, wherein R' and R'' are independently selected from the group consisting of H, halogen, $-OH$, OMe , $-NH_2$, $-NO_2$, $-CN$, $-CF_3$, $-OCF_3$, $-N_3$, $-S(=O)_2C_1-C_4alkyl$, $-S(=O)C_1-$

C₄alkyl, -S-C₁-C₄alkyl, -OS(=O)₂CF₃, Ph, -NHCH₂Ph, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1 or 3. In some cases, R' is H or OMe, and R'' is H.

[00230] In other embodiments, the present invention provides compounds of formula I-j, wherein R₁₇ is -NR₁₅R₁₆ or -OR₁₅. In certain other embodiments, R₁₇ is -OH, -OMe, -NEt, -NHEt, -NHPh, -NH₂, or -NHCH₂pyridyl.

[00231] In another embodiment, the present invention provides compounds of formula I-k:



wherein R' and R'' are independently selected from the group consisting of H, halogen, -OH, -NH₂, -NO₂, -CN, -CF₃, -OCF₃, -N₃, -SO₃H, -S(=O)₂alkyl, -S(=O)alkyl, -OS(=O)₂CF₃, acyl, alkyl, alkoxyl, alkylamino, alkylthio, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino; and wherein each acyl, alkyl, alkoxyl, alkylamino, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio may be substituted or unsubstituted;

R₁₈ is selected from the group consisting of -NR₁₅R₁₆, -C(=O)NR₁₅R₁₆, -(C=O)OR₁₅, -OR₁₅, alkyl, aryl, cycloalkyl, heterocyclyl, and at one labeling group; wherein each alkyl, aryl, cycloalkyl, and heterocyclyl may be substituted or unsubstituted;

wherein p is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10;

and n is 0, 1, or 2;

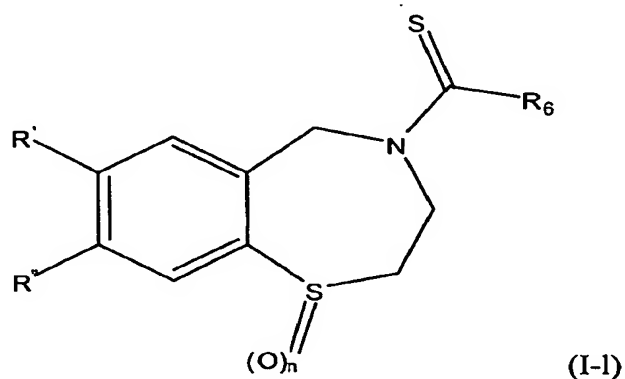
and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

[00232] In certain embodiments, the present invention provides compounds of formula I-k, wherein R' and R'' are independently selected from the group consisting of H,

halogen, -OH, OMe, -NH₂, -NO₂, -CN, -CF₃, -OCF₃, -N₃, -S(=O)₂C₁-C₄alkyl, -S(=O)C₁-C₄alkyl, -S-C₁-C₄alkyl, -OS(=O)₂CF₃, Ph, -NHCH₂Ph, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1 or 3. In some cases, R' is H or OMe, and R'' is H.

[00233] In other embodiments, the present invention provides compounds of formula **I-k**, wherein R₁₈ is selected from the group consisting of -NR₁₅R₁₆, -(C=O)OR₁₅, -OR₁₅, alkyl, aryl, and at one labeling group; and wherein each alkyl and aryl may be substituted or unsubstituted. In some cases, m is 1, and R₁₈ is Ph, C(=O)OMe, C(=O)OH, aminoalkyl, NH₂, NHOH, or NHCbz. In other cases, m is 0, and R₁₈ is C₁-C₄ alkyl, such as Me, Et, propyl, and butyl. In yet other cases, m is 2, and R₁₈ is pyrrolidine, piperidine, piperazine, or morpholine. In some embodiments, m is 3, 4, 5, 6, 7, or 8, and R₁₈ is a fluorescent labeling group selected from bodipy, dansyl, fluorescein, rhodamine, Texas red, cyanine dyes, pyrene, coumarins, Cascade Blue™, Pacific Blue, Marina Blue, Oregon Green, 4',6-Diamidino-2-phenylindole (DAPI), indopyra dyes, lucifer yellow, propidium iodide, porphyrins, arginine, and variants and derivatives thereof.

[00234] In yet another embodiment, the present invention provides compounds of formula of **I-l**:



wherein R' and R'' are independently selected from the group consisting of H, halogen, -OH, -NH₂, -NO₂, -CN, -CF₃, -OCF₃, -N₃, -SO₃H, -S(=O)₂alkyl, -S(=O)alkyl, -OS(=O)₂CF₃, acyl, alkyl, alkoxyl, alkylamino, alkylthio, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)aryl amino; and wherein each acyl, alkyl, alkoxyl, alkylamino, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio may be substituted or unsubstituted;

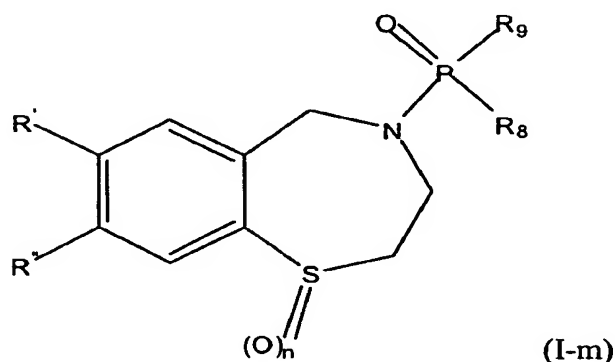
R₆ and n are as defined in compounds of formula **I-a**;

and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

[00235] In certain embodiments, the present invention provides compounds of formula **I-l**, wherein R' and R'' are independently selected from the group consisting of H, halogen, -OH, OMe, -NH₂, -NO₂, -CN, -CF₃, -OCF₃, -N₃, -S(=O)₂C₁-C₄alkyl, -S(=O)C₁-C₄alkyl, -S-C₁-C₄alkyl, -OS(=O)₂CF₃, Ph, -NHCH₂Ph, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1 or 3. In some cases, R' is H or OMe, and R'' is H.

[00236] In other embodiments, the present invention provides compounds of formula **I-l**, wherein R₆ is selected from the group consisting of -NR₁₅R₁₆, -NHNHNR₁₅R₁₆, -OR₁₅, -NHOH, -CH₂X, acyl, alkenyl, alkyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkenyl, alkyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted. In some cases, R₆ is -NR₁₅R₁₆ such as -NHPh, pyrrolidine, piperidine, piperazine, morpholine, and the like. In some other cases, R₆ is alkoxyl, such as -O-tBu.

[00237] In a further embodiment, the present invention provides compounds of formula **I-m**:



wherein R' and R'' are independently selected from the group consisting of H, halogen, -OH, -NH₂, -NO₂, -CN, -CF₃, -OCF₃, -N₃, -SO₃H, -S(=O)₂alkyl, -S(=O)alkyl, -OS(=O)₂CF₃, acyl, alkyl, alkoxyl, alkylamino, alkylthio, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino; and wherein each acyl, alkyl, alkoxyl, alkylamino, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio may be substituted or unsubstituted;

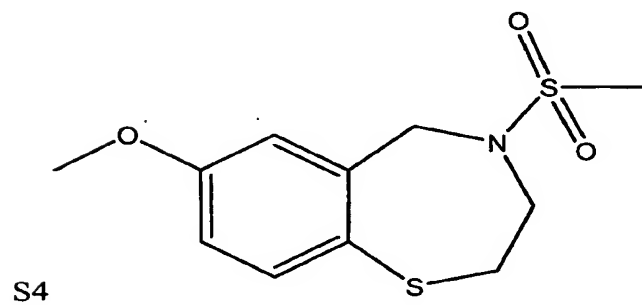
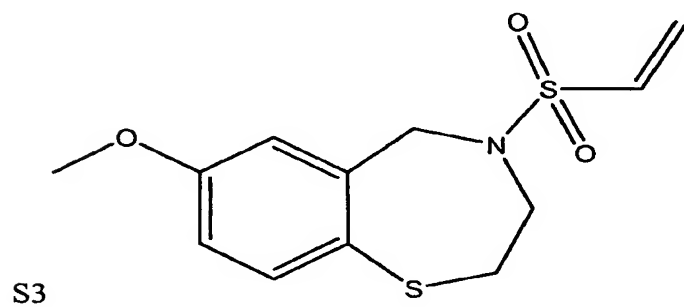
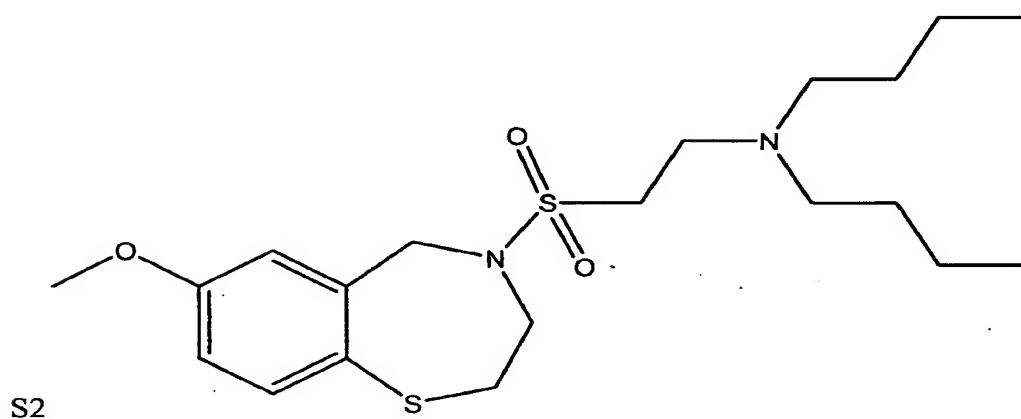
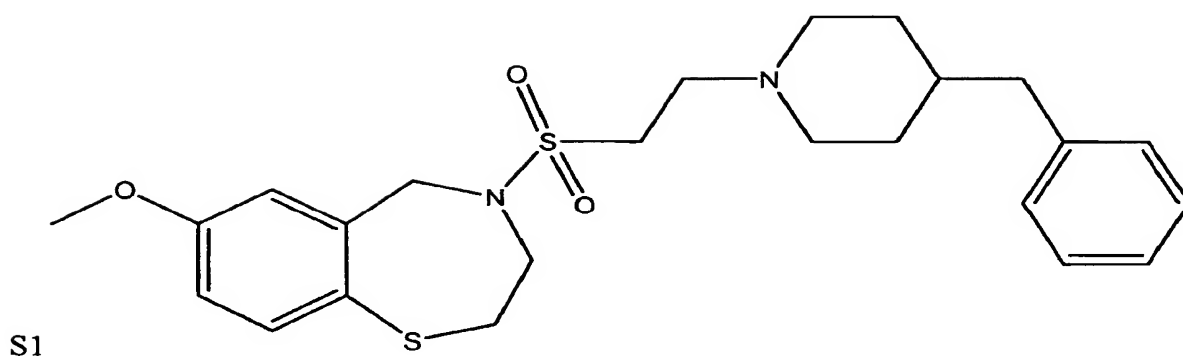
R₈, R₉ and n are as defined in compounds of formula **I-a** above; and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

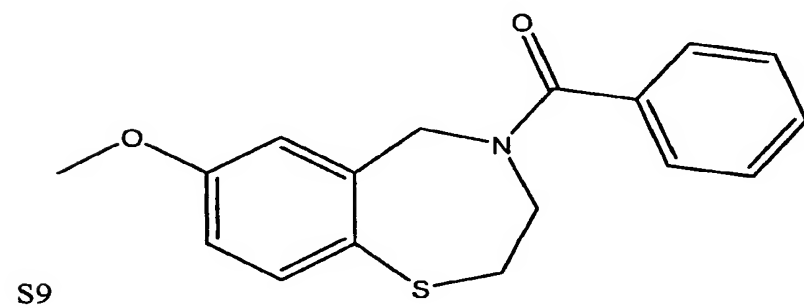
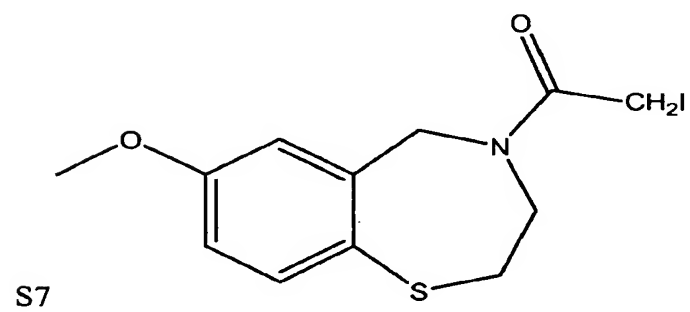
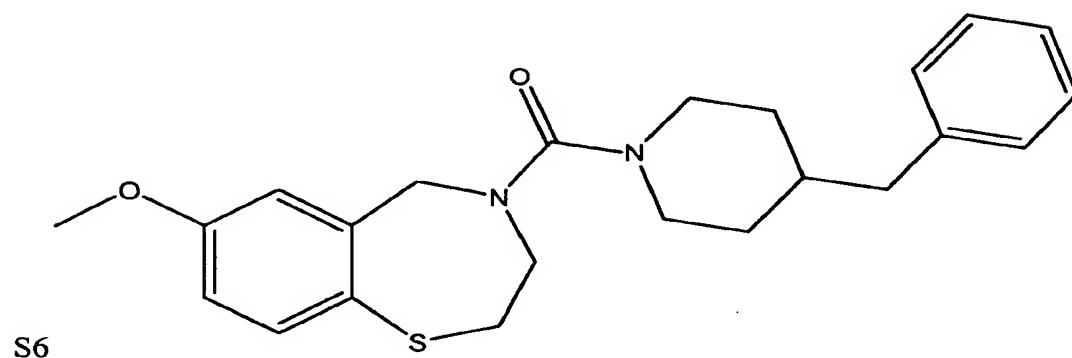
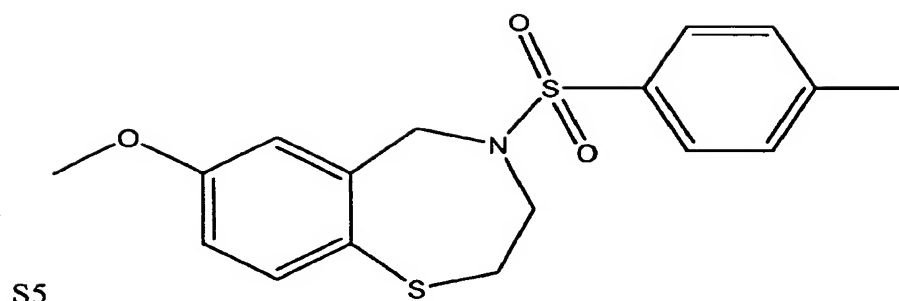
[00238] In certain embodiments, the present invention provides compounds of formula **I-m**, wherein R' and R'' are independently selected from the group consisting of H, halogen, -OH, OMe, -NH₂, -NO₂, -CN, -CF₃, -OCF₃, -N₃, -S(=O)₂C₁-C₄alkyl, -S(=O)C₁-C₄alkyl, -S-C₁-C₄alkyl, -OS(=O)₂CF₃, Ph, -NHCH₂Ph, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1 or 3. In some cases, R' is H or OMe, and R'' is H.

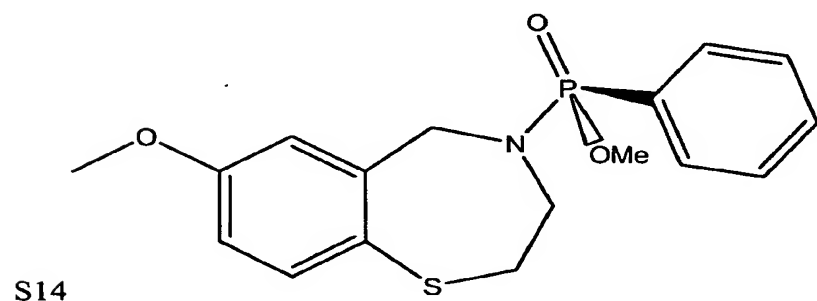
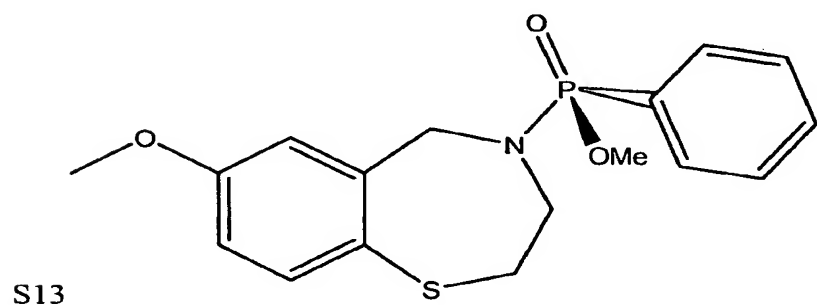
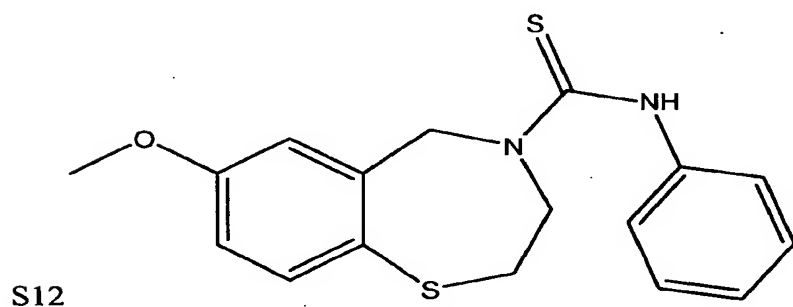
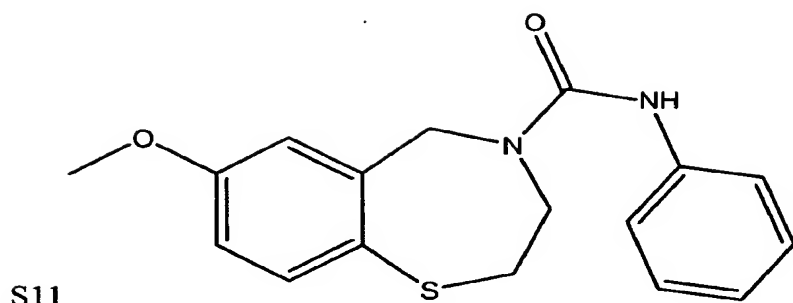
[00239] In other embodiments, the present invention provides compounds of formula **I-m**, wherein R₈ and R₉ are independently alkyl, aryl, -OH, alkoxyl, or alkylamino. In some cases, R₈ is C₁-C₄ alkyl such as Me, Et, propyl and butyl; and R₉ is aryl such as phenyl.

[00240] The compounds of the invention may optionally comprise a labeling group, such as a fluorescent, bioluminescent, chemiluminescent, colorimetric or radioactive labeling group. Suitable fluorescent labeling groups include, but are not limited to, bodipy, dansyl, fluorescein, rhodamine, Texas red, cyanine dyes, pyrene, coumarins, Cascade BlueTM, Pacific Blue, Marina Blue, Oregon Green, 4', 6-Diamidino-2-phenylindole (DAPI), indopyra dyes, lucifer yellow, propidium iodide, porphyrins, and variants and derivatives thereof. One of skill in the art can readily select a suitable marker or labeling group, and conjugate such a labeling group to any of the compounds of the invention, without undue experimentation.

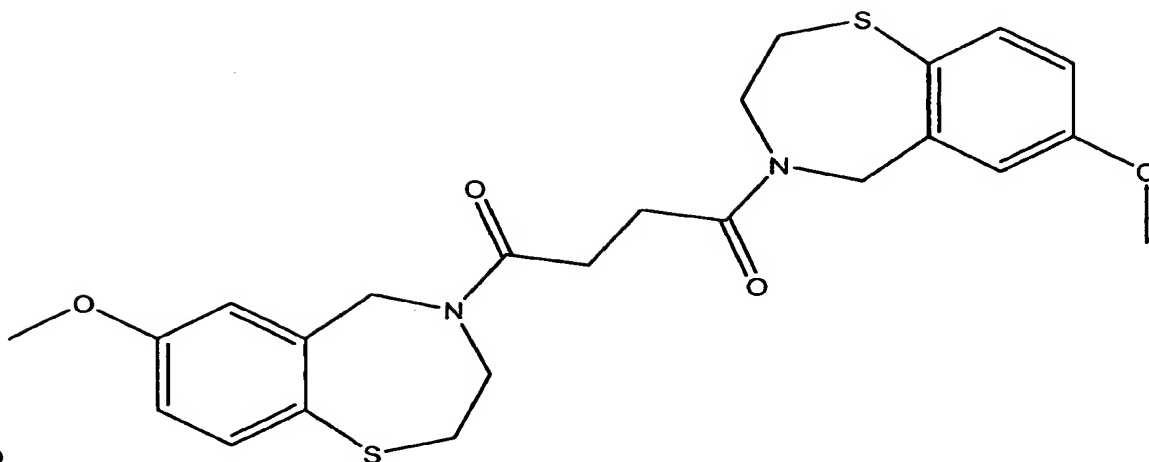
[00241] The compounds of Formula I treat and prevent disorders and diseases associated with the RyR receptors. Examples of such compounds include, without limitation, S1, S2, S3, S4, S5, S6, S7, S9, S11, S12, S13, S14, S19, S20, S22, S23, S25, S26, S36, S37, S38, S40, S43, S44, S45, S46, S47, S48, S49, S50, S51, S52, S53, S54, S55, S56, S57, S58, S59, S60, S61, S62, S63, S64, S66, S67, S68, S69, S70, S71, S72, S73, S74, S75, S76, S77, S78, S79, S80, S81, S82, S83, S84, S85, S86, S87, S88, S89, S90, S91, S92, S93, S94, S95, S96, S97, S98, S99, S100, S101, S102, S103, S104, S105, S107, S108, S109, S110, S111, S112, S113, S114, S115, S116, S117, S118, S119, S120, S121, S122, and S123., which have the following structures:



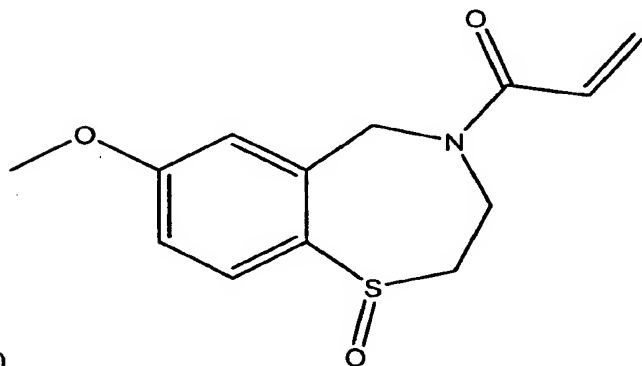




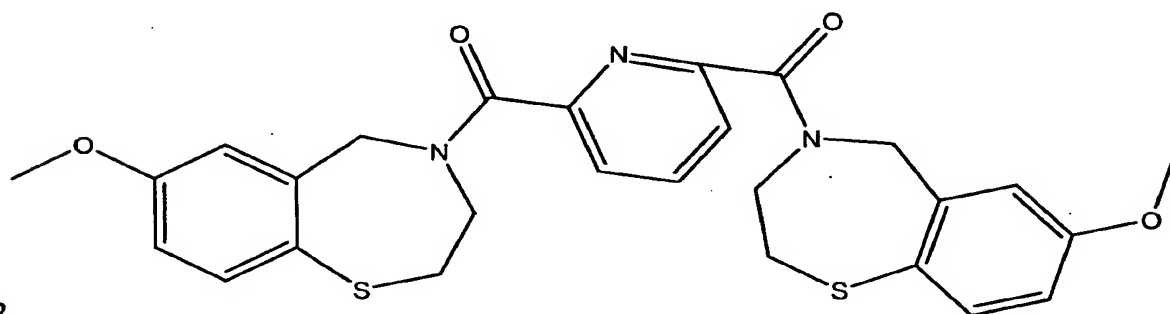
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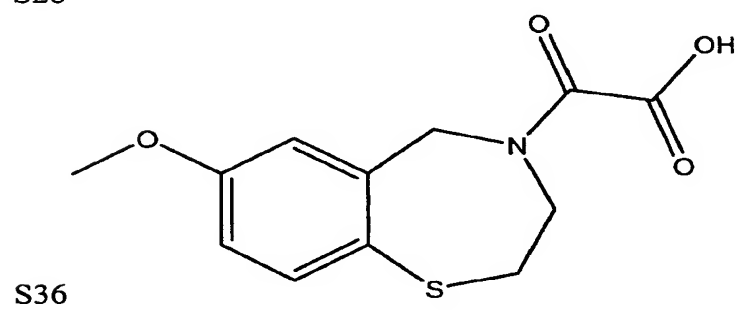
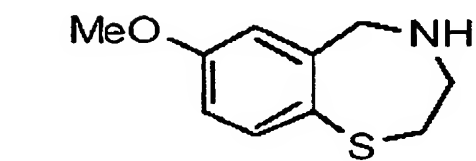
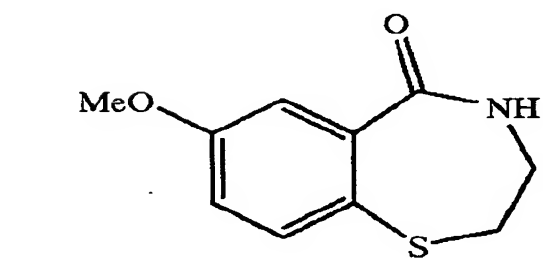
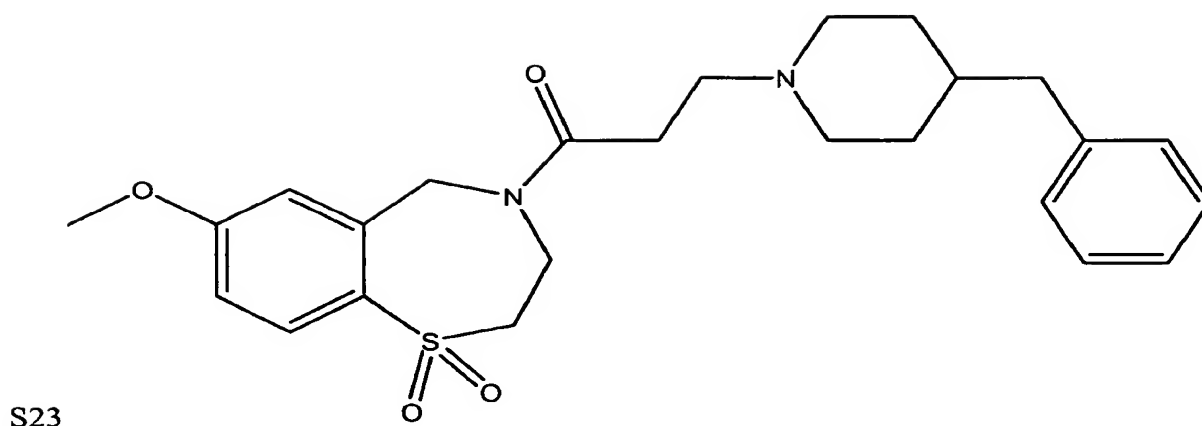


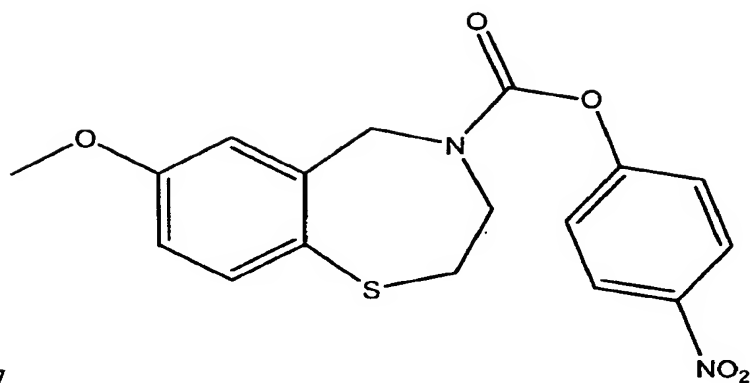
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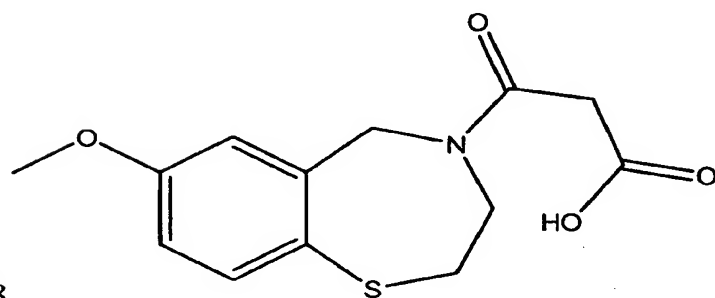
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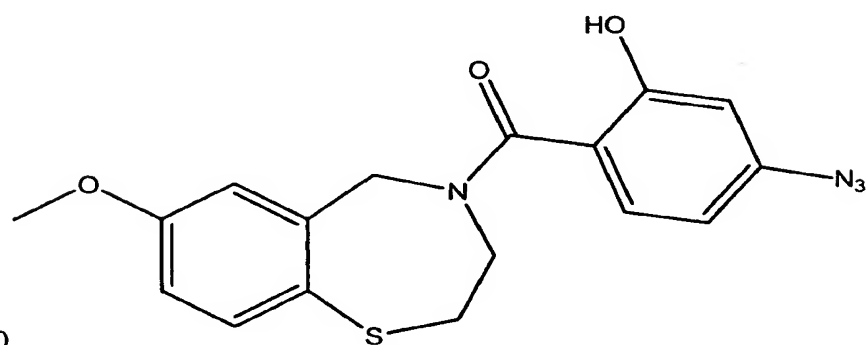




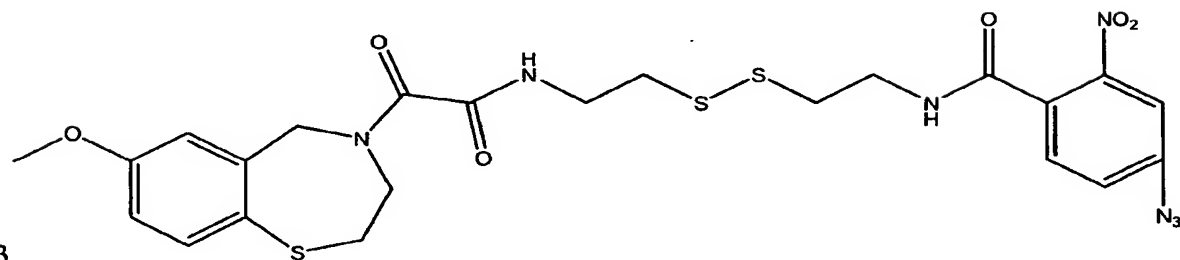
S37



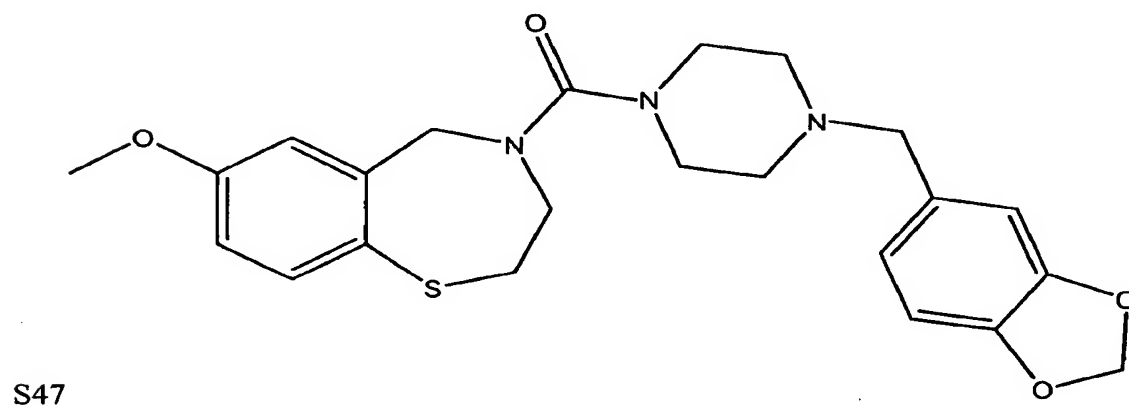
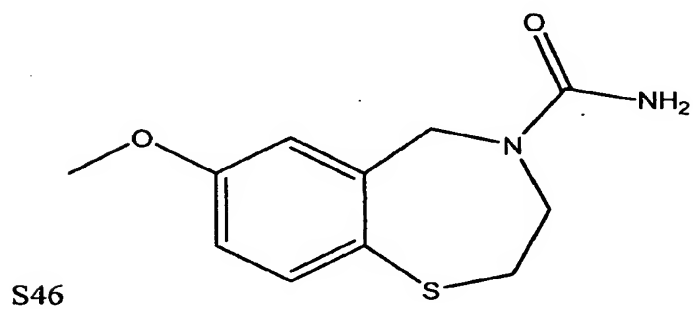
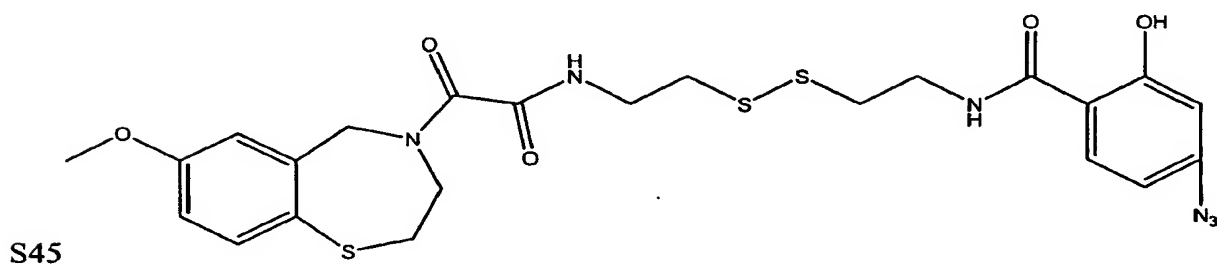
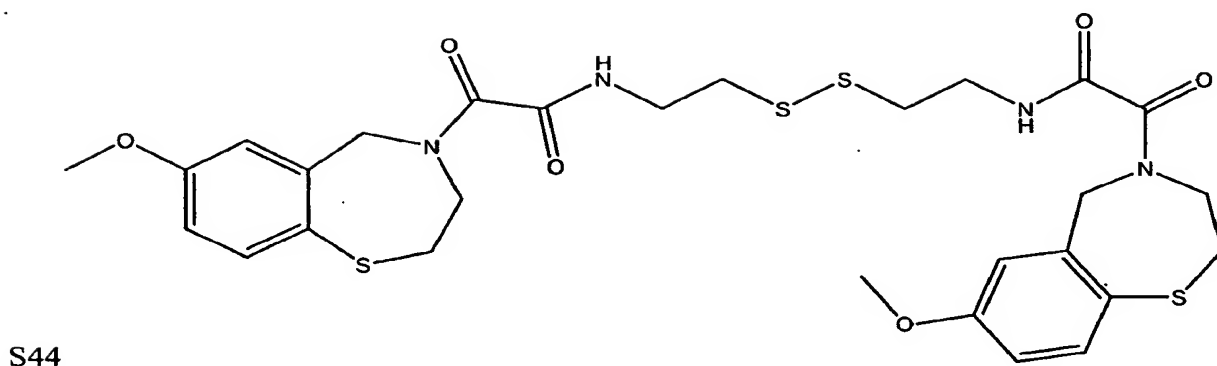
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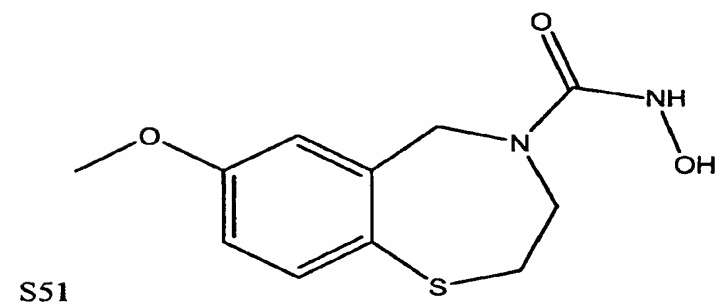
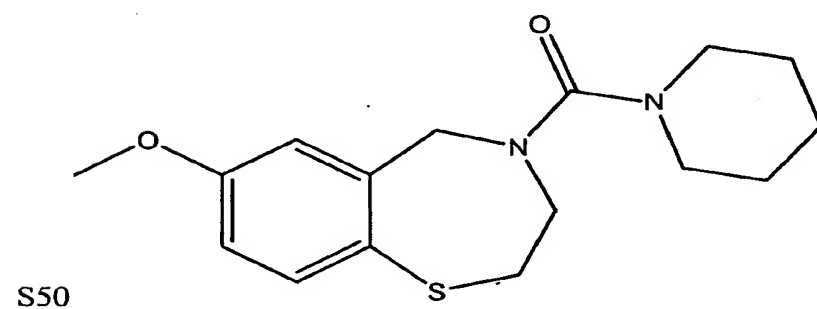
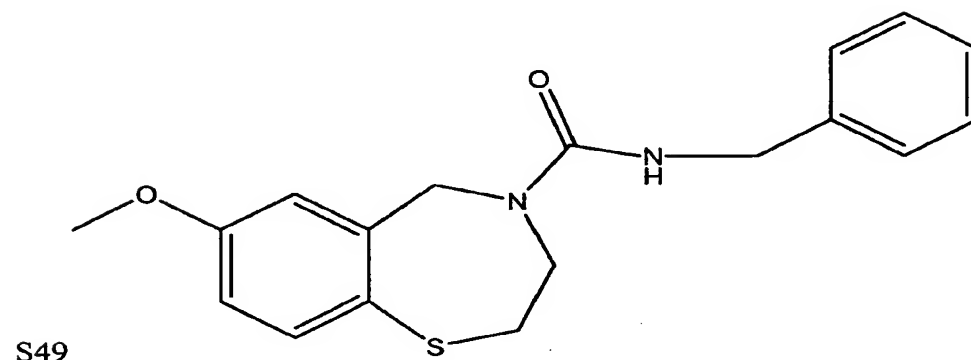
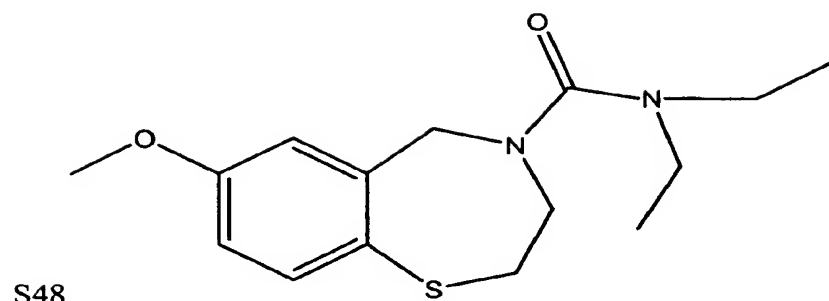


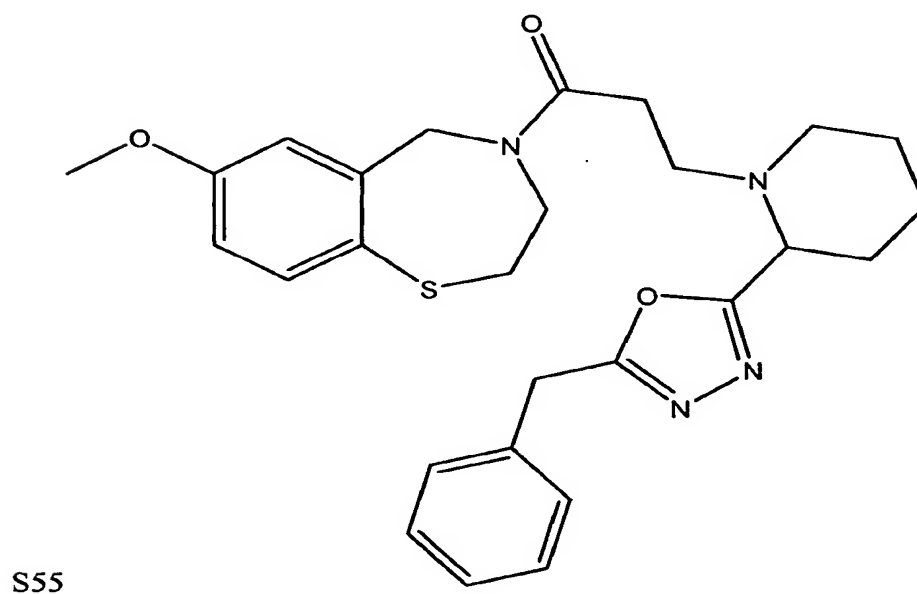
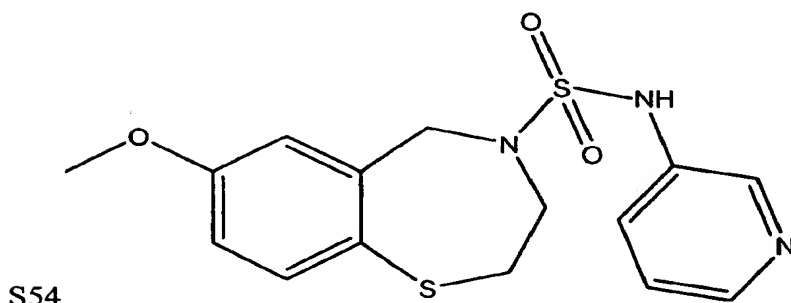
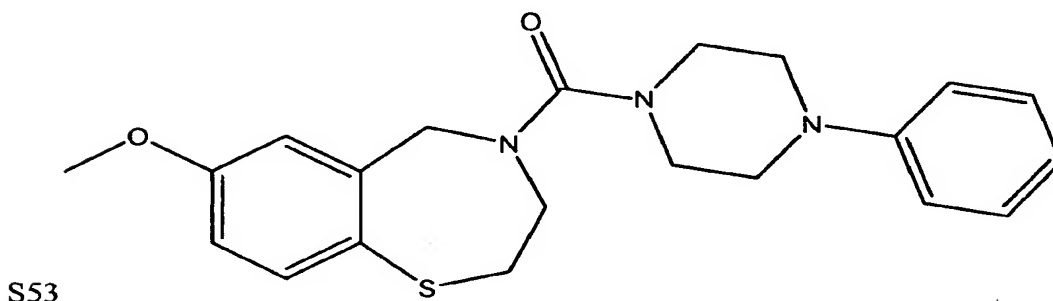
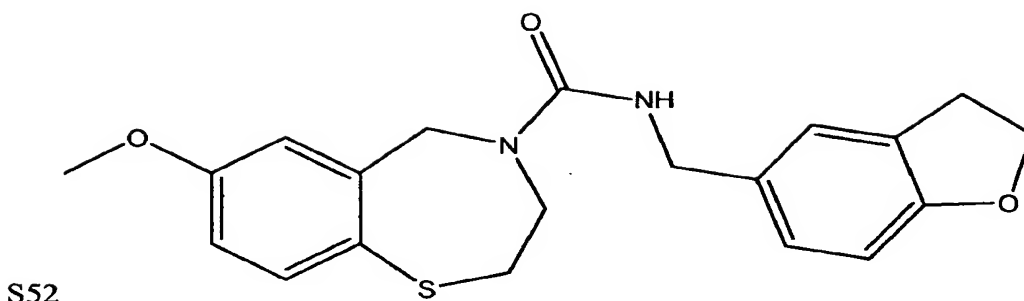
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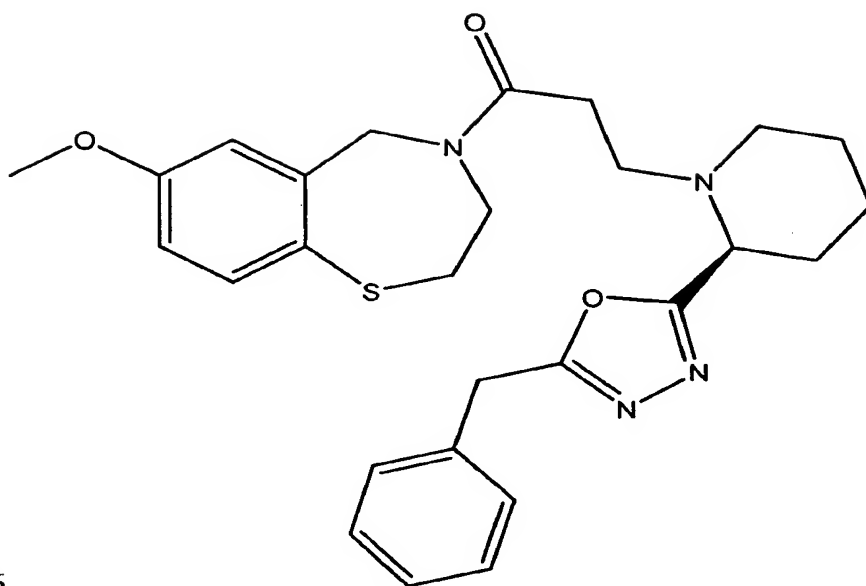


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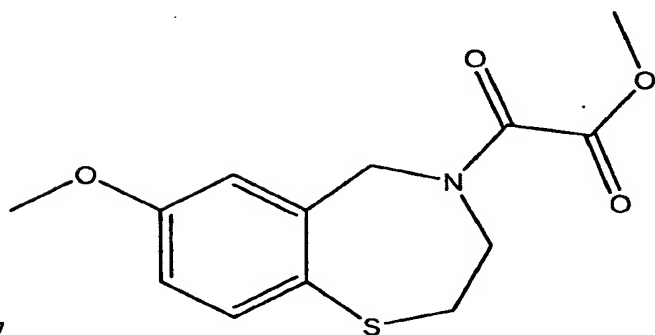




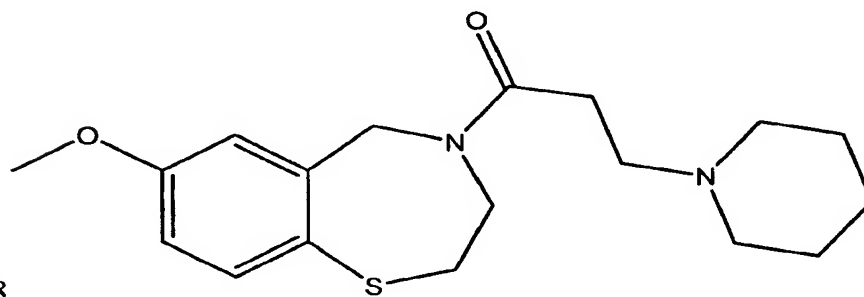




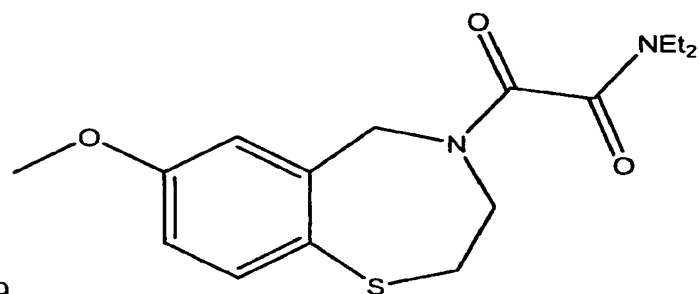
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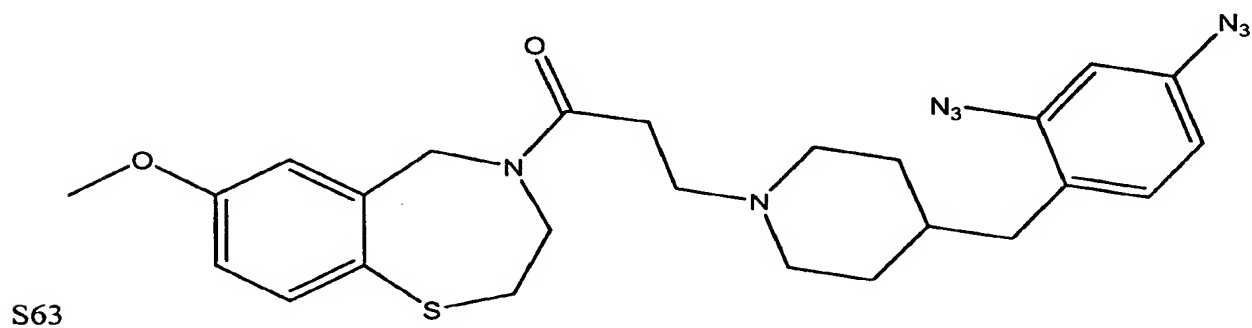
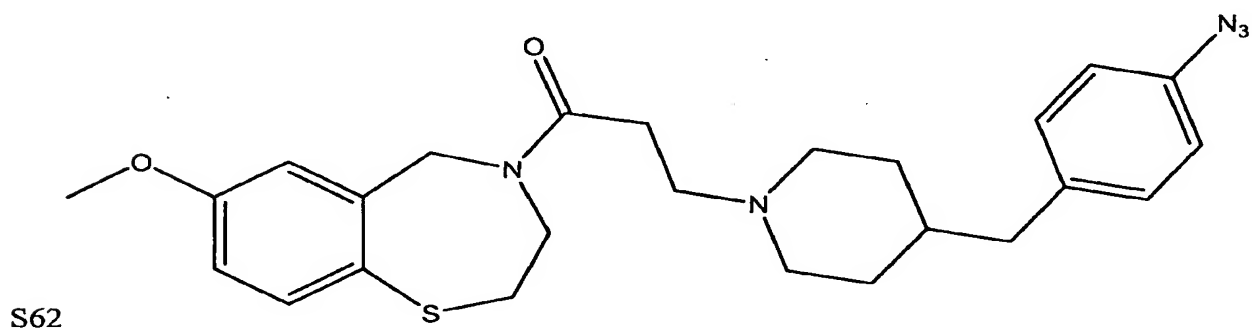
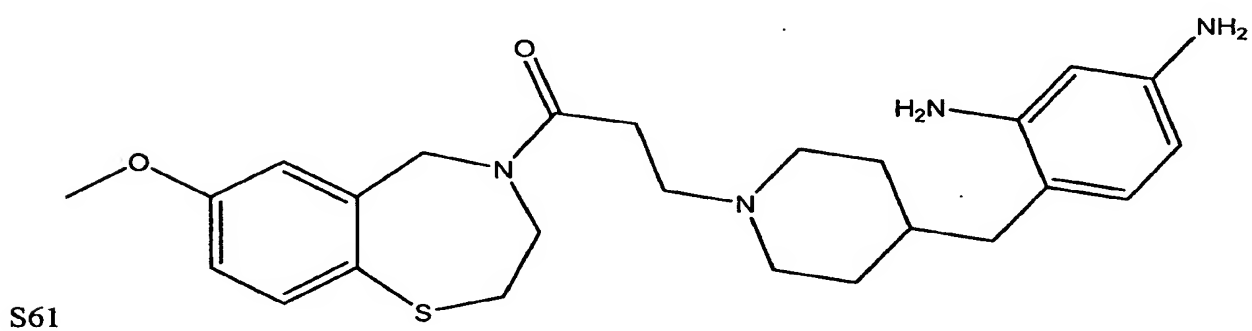
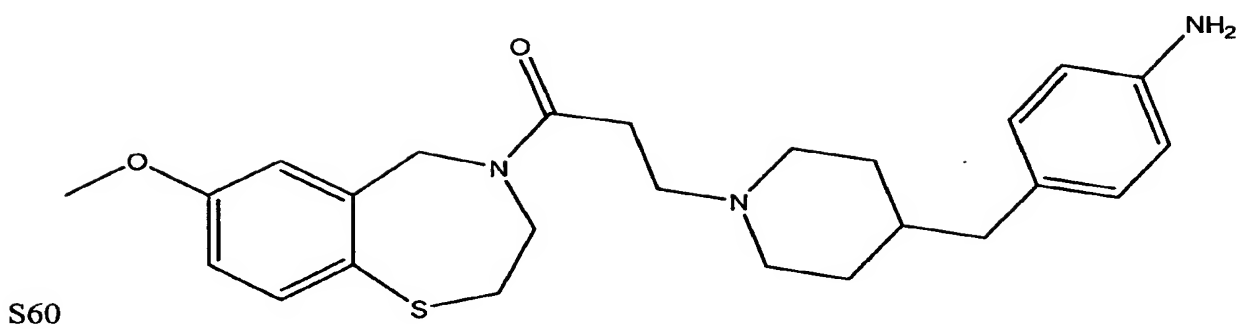
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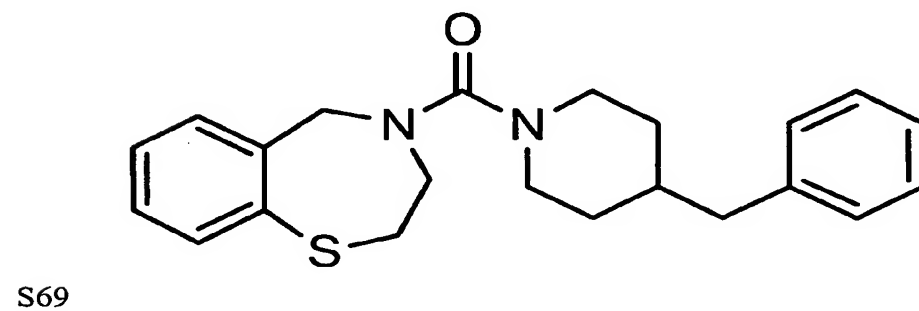
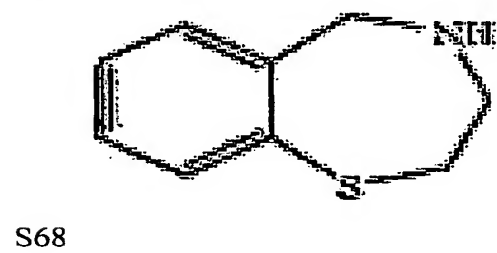
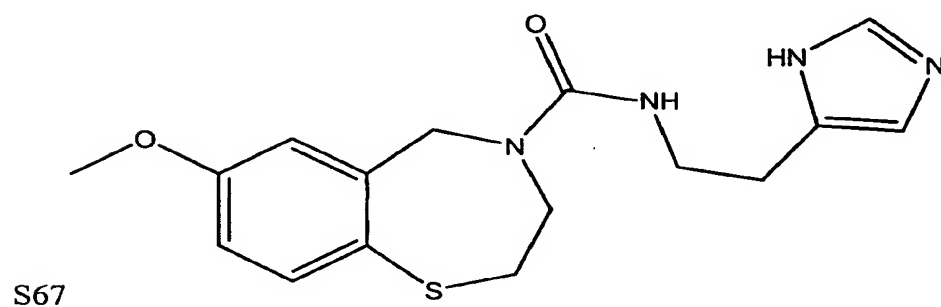
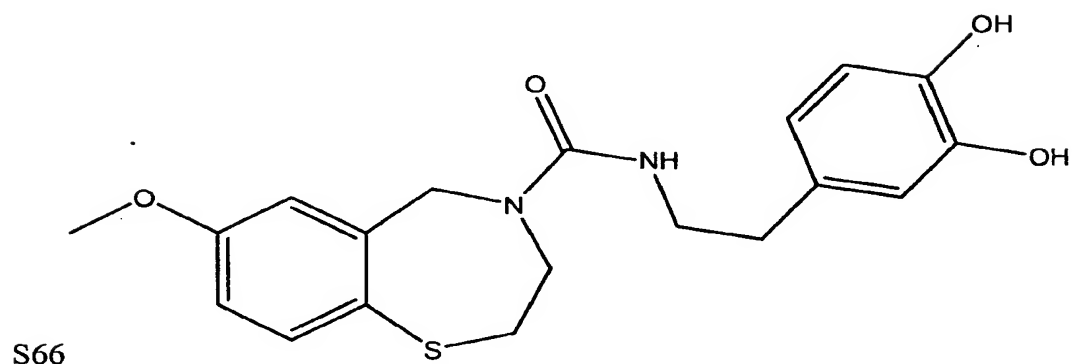
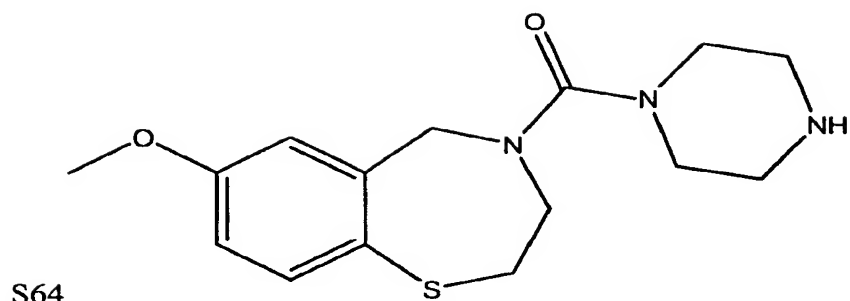


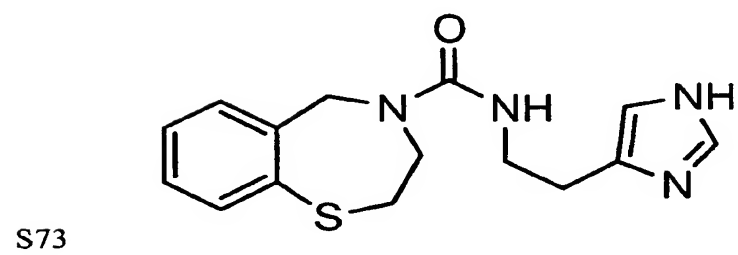
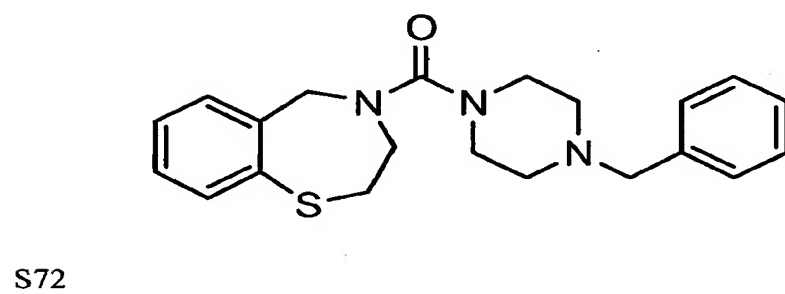
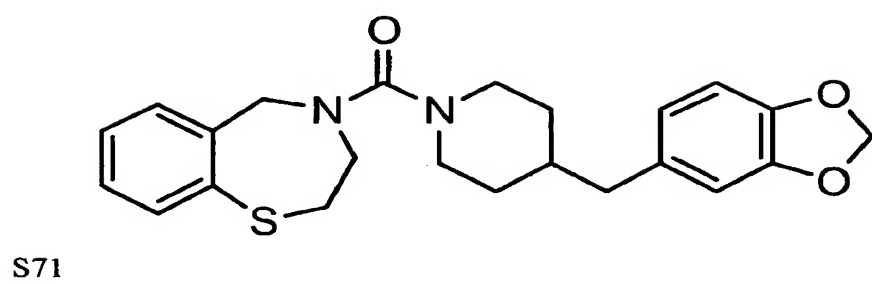
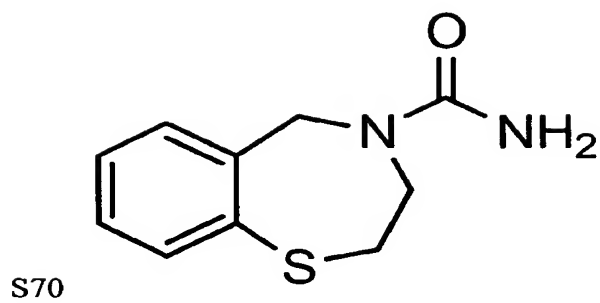
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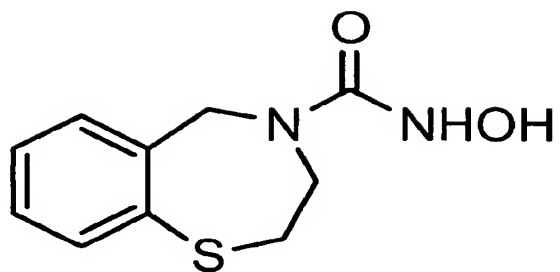


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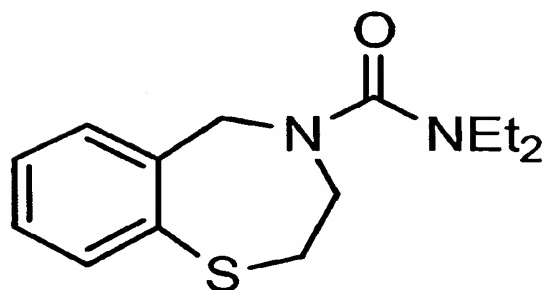




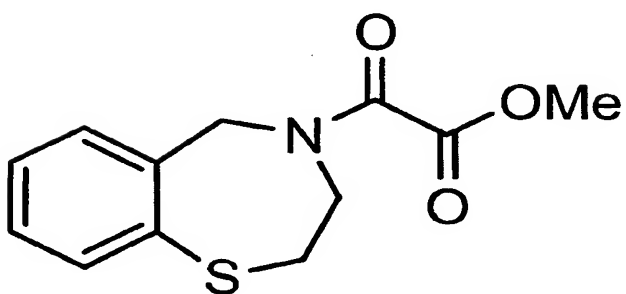




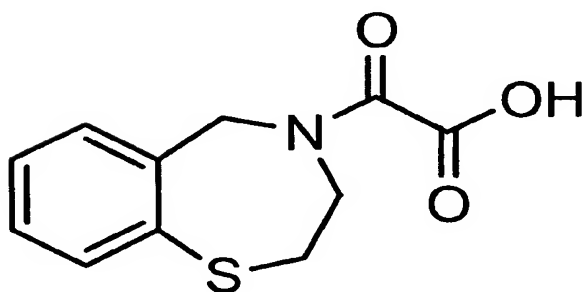
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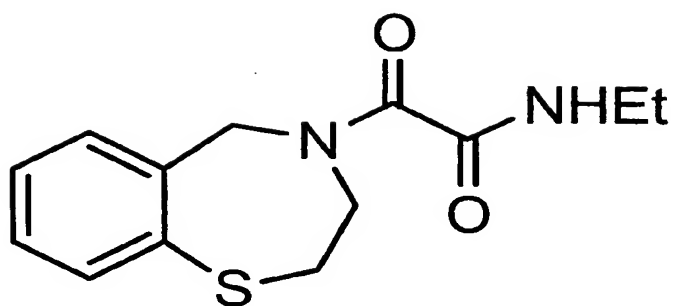
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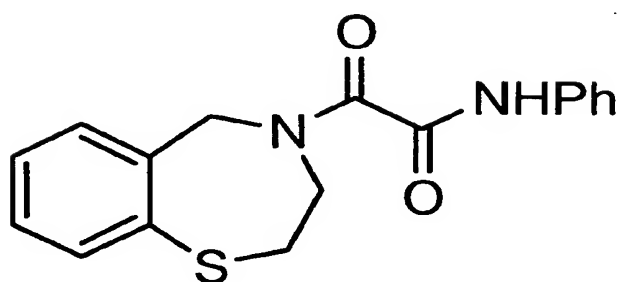
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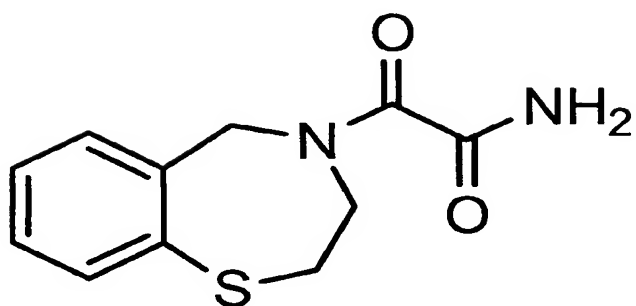
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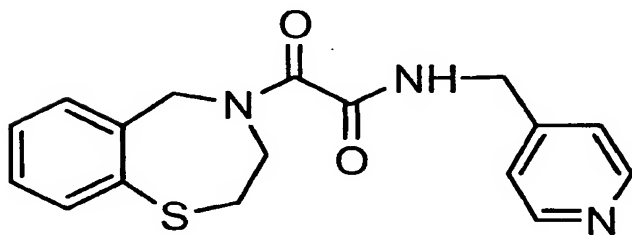
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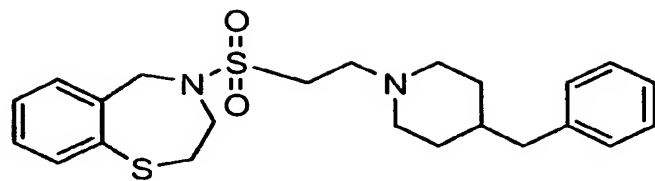
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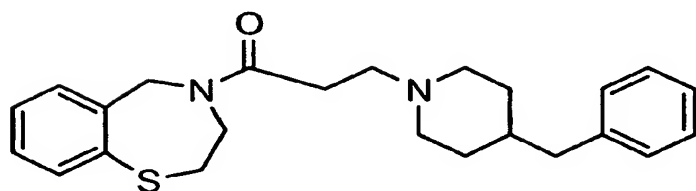
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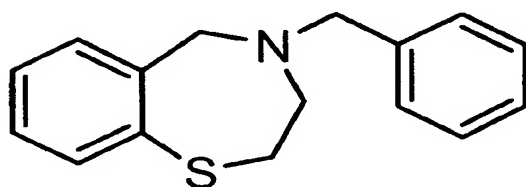
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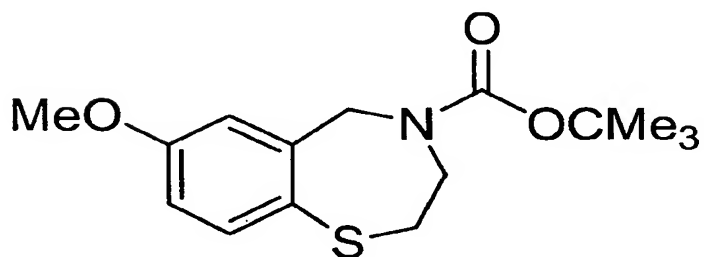
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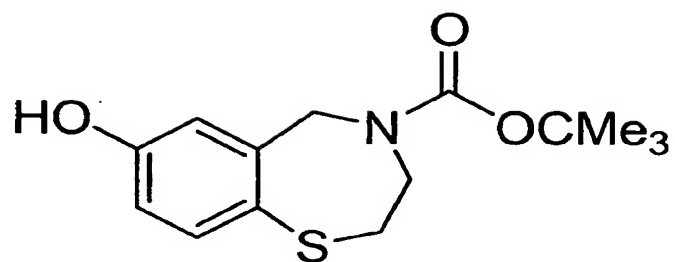
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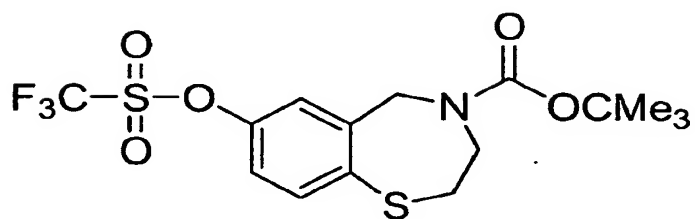
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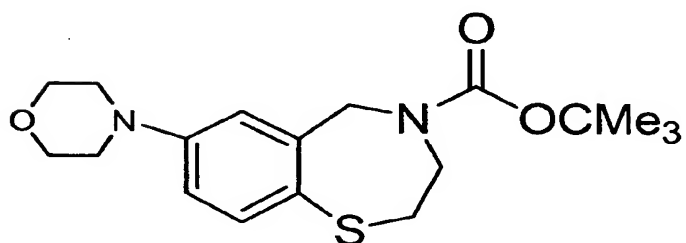
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S86



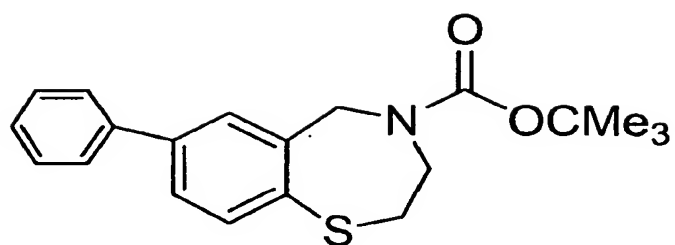
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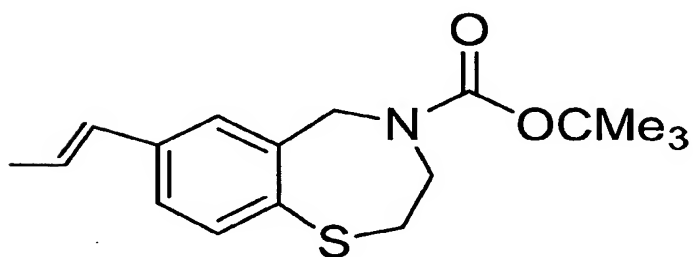
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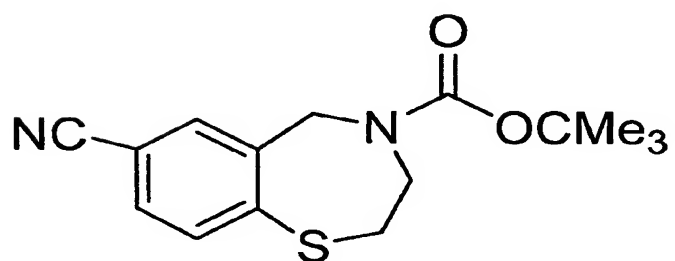
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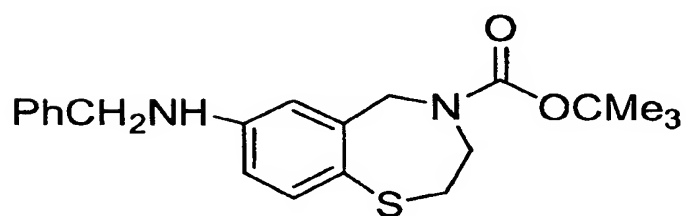
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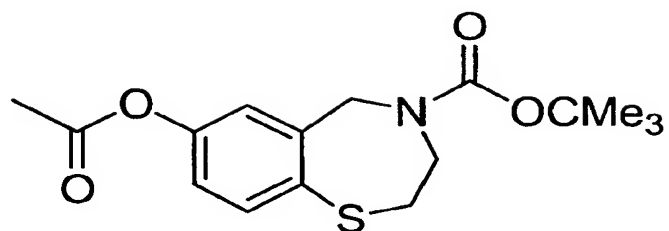
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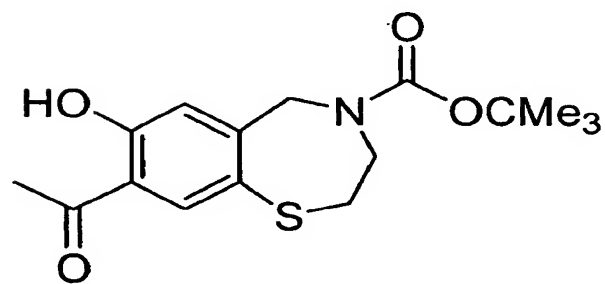
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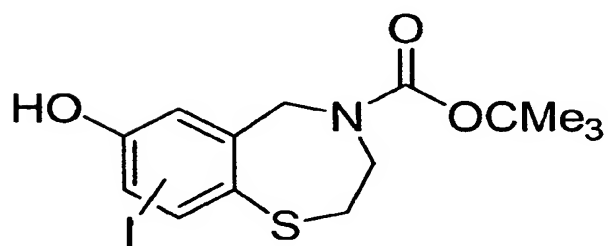
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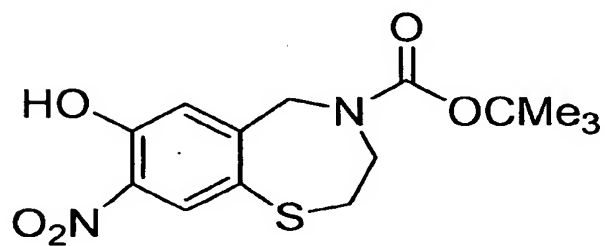
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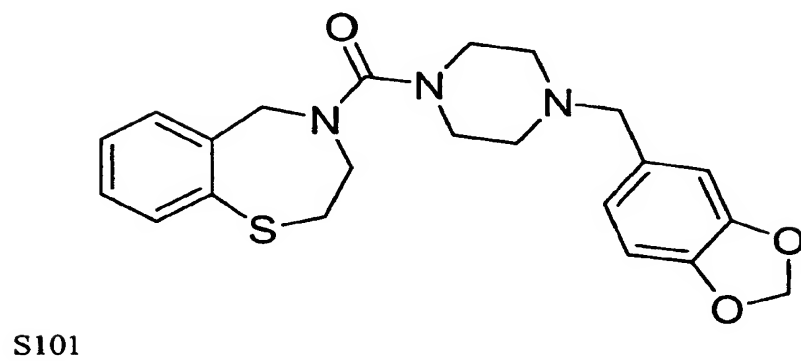
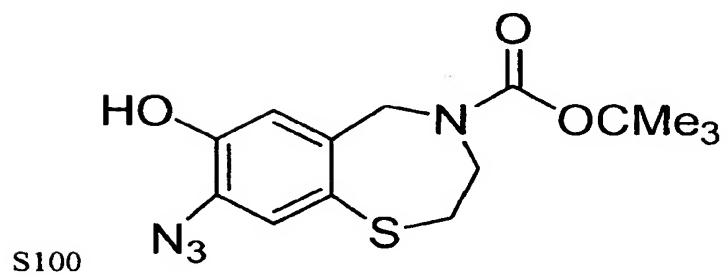
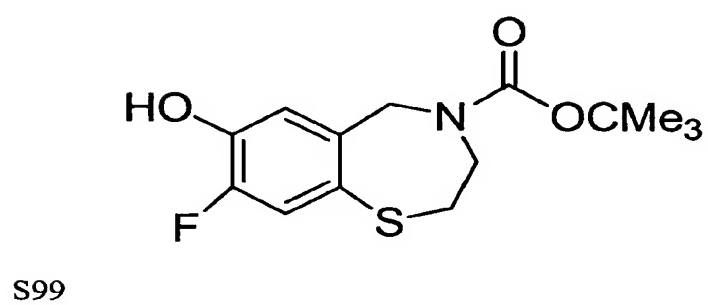
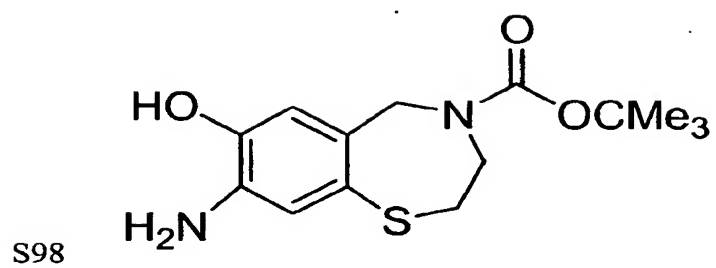
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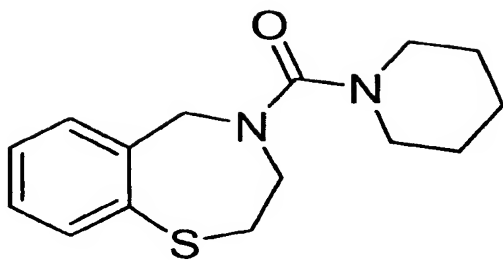


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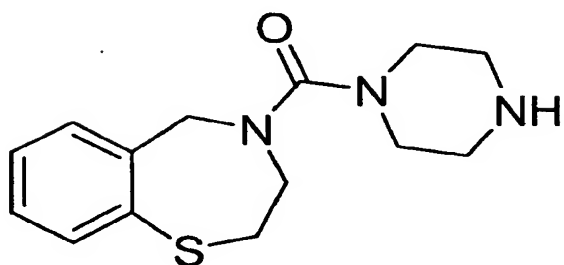


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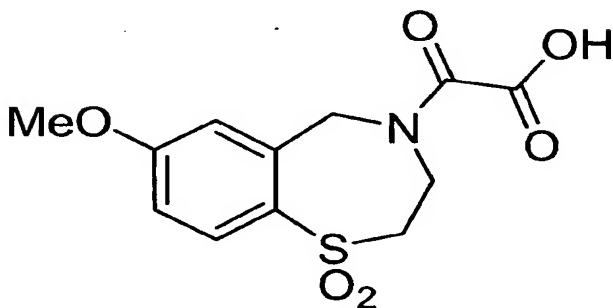




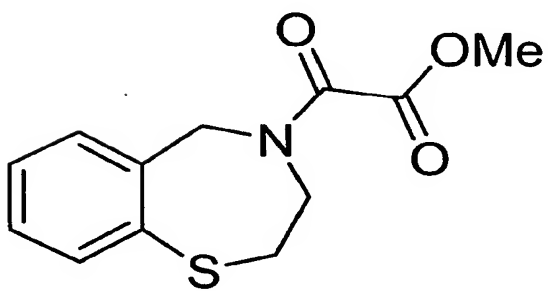
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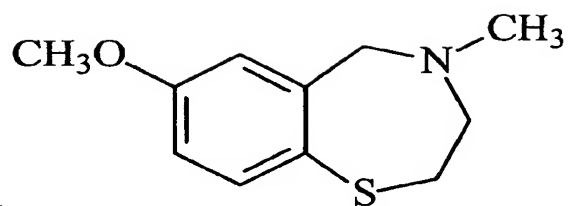
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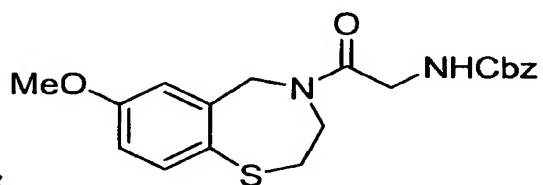
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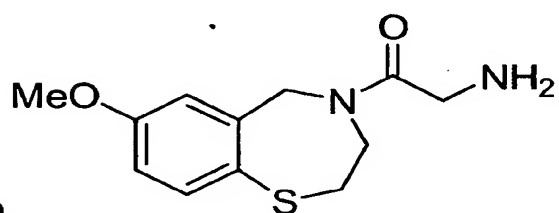
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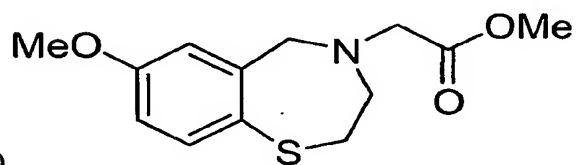
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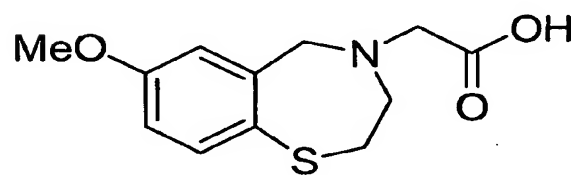
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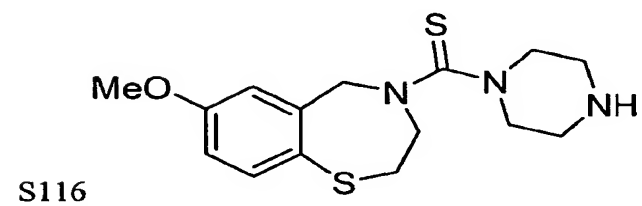
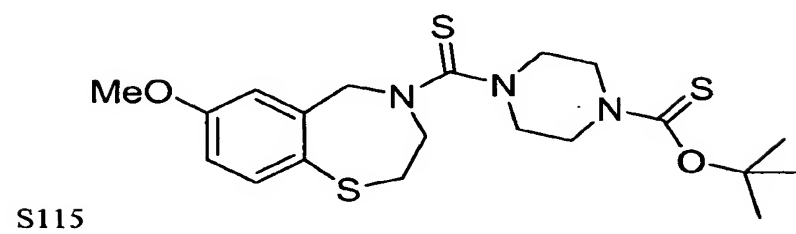
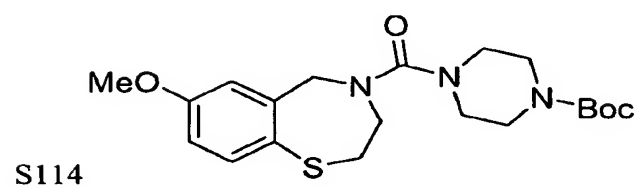
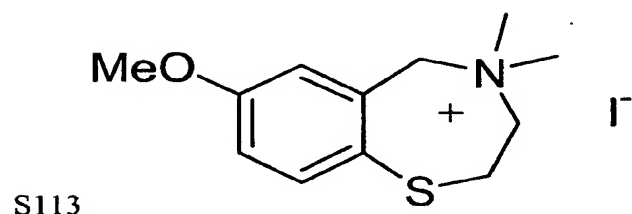
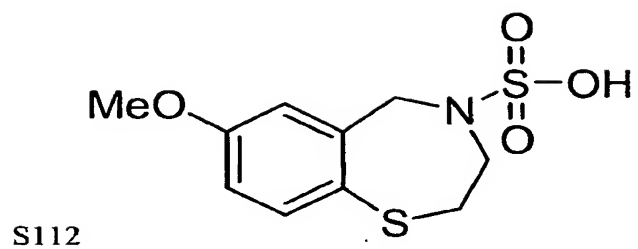
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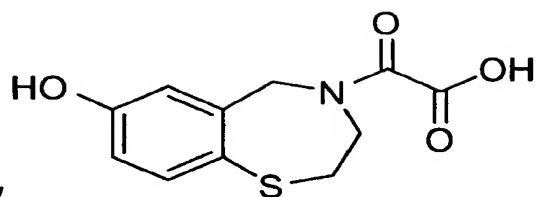


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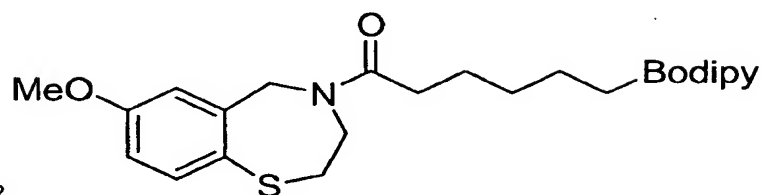


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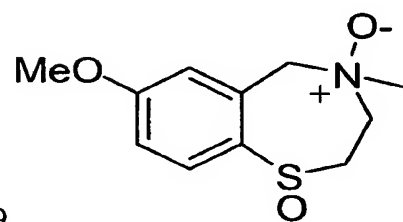




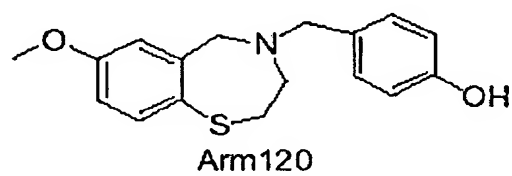
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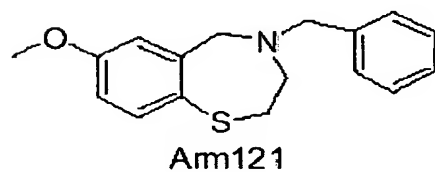


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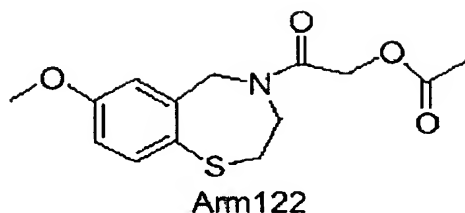
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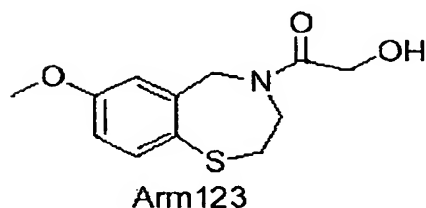


Arm121

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[00242] In an embodiment of the present invention, if R_2 is $C=O(R_5)$ or SO_2R_7 , then R is at positions 2, 3, or 5 on the benzene ring.

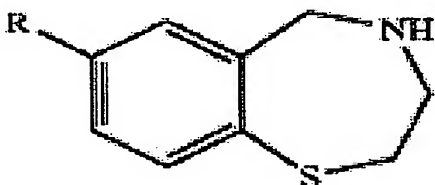
[00243] In another embodiment of the invention, if R_2 is $C=O(R_5)$ or SO_2R_7 , then each R is independently selected from the group consisting of H, halogen, -OH, -NH₂, -NO₂, -CN, -N₃, -SO₃H, acyl, alkyl, alkylamino, cycloalkyl, heterocyclyl, heterocyclylalkyl, alkenyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino; wherein each acyl, alkyl, alkoxyl, alkylamino, cycloalkyl, heterocyclyl, heterocyclylalkyl, alkenyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino may be substituted with one or more radicals independently selected from the group consisting of halogen, N, O, -S-, -CN, -N₃, -SH, nitro, oxo, acyl, alkyl, alkoxyl, alkylamino, alkenyl, aryl, (hetero-)cycloalkyl, and (hetero-)cyclyl.

[00244] In another embodiment of the invention, if R_2 is $C=O(R_5)$ or SO_2R_7 , then there are at least two R groups attached to the benzene ring. Furthermore, there are at least two R groups attached to the benzene ring, and both R groups are attached at positions 2, 3, or 5 on the benzene ring. Still furthermore, each R is independently selected from the group consisting of H, halogen, -OH, -NH₂, -NO₂, -CN, -N₃, -SO₃H, acyl, alkyl, alkylamino, cycloalkyl, heterocyclyl, heterocyclylalkyl, alkenyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino; wherein each acyl, alkyl, alkoxyl, alkylamino, cycloalkyl, heterocyclyl, heterocyclylalkyl, alkenyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino may be

substituted with one or more radicals independently selected from the group consisting of halogen, N, O, -S-, -CN, -N₃, -SH, nitro, oxo, acyl, alkyl, alkoxyl, alkylamino, alkenyl, aryl, (hetero-)cycloalkyl, and (hetero-)cyclyl.

[00245] In another embodiment of the invention, if R₂ is C=O(R₅), then R₅ is selected from the group consisting of -NR₁₆, NHNHR₁₆, NHOH, -OR₁₅, CONH₂NHR₁₆, CONR₁₆, CH₂X, acyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted with one or more radicals independently selected from the group consisting of halogen, N, O, -S-, -CN, -N₃, nitro, oxo, acyl, alkyl, alkoxyl, alkylamino, alkenyl, aryl, (hetero-)cycloalkyl, and (hetero-)cyclyl.

[00246] In another embodiment, the present invention provides veterinary uses of compounds of Formula II:



wherein R=OR', SR', NR', alkyl, or halide and R' = alkyl, aryl, or H, and wherein R can be at position 2, 3, 4, or 5. Formula II is discussed also in co-pending application 10/680,988, the disclosure of which is incorporated herein in its entirety by reference.

Routes of Activity

[00247] The compounds of the invention reduce the open probability of RyR by increasing the affinity of FKBP12 (calstabin1) and FKBP12.6 (calstabin2) for, respectively PKA-phosphorylated RyR1 and PKA-phosphorylated RyR2. Moreover, the compounds of the invention normalize gating of mutant RyR channels, including CPVT-associated mutant RyR2 channels, by increasing FKBP12 (calstabin1) and FKBP12.6 (calstabin2) binding affinity. Therefore, the compounds of the invention prevent disorders and conditions involving modulation of the RyR receptors, particularly the RyR1 and RyR2 receptors, including but not limited to disorders and conditions involving the interaction of the calstabin protein to the RyR receptor in the RyR-calstabin protein complex. Examples of such disorders and conditions include, without limitation, cardiac conditions, disorders and

diseases, skeletal muscular disorders and diseases, cognitive disorders and diseases, malignant hyperthermia, diabetes, and sudden infant death syndrome. Cardiac conditions, disorder and diseases include, but are not limited to, irregular heartbeat disorders and diseases; exercise-induced irregular heartbeat disorders and diseases; sudden cardiac death; exercise-induced sudden cardiac death; congestive heart failure; chronic obstructive pulmonary disease; and high blood pressure. Irregular heartbeat disorders and diseases include exercise-induced irregular heartbeat disorders and diseases that include, but are not limited to, atrial and ventricular arrhythmia; atrial and ventricular fibrillation; atrial and ventricular tachyarrhythmia; atrial and ventricular tachycardia; catecholaminergic polymorphic ventricular tachycardia (CPVT), arrhythmogenic right ventricular cardiomyopathy (ARVC), and exercise-induced variants thereof. Skeletal muscular disorder and diseases include, but are not limited to, skeletal muscle fatigue, exercise-induced skeletal muscle fatigue, muscular dystrophy, bladder disorders, and incontinence. Cognitive disorders and diseases include, but are not limited to, Alzheimer's Disease, forms of memory loss, and age-dependent memory loss. The compounds of the invention treat these disorders and conditions by increasing FKBP12 (calstabin1)-RyR1 binding affinity and increasing FKBP12.6 (calstabin2)-RyR2 binding affinity.

[00248] In accordance with the foregoing, the present invention provides a method for limiting or preventing a decrease in the level of RyR-bound FKBP (calstabin) in cells of a subject. As used herein, "RyR" includes RyR1, RyR2, and RyR3. Additionally, FKBP includes both FKBP12 (calstabin1) and FKBP12.6 (calstabin2). "RyR-bound FKBP" therefore refers to RyR1-bound FKBP12 (calstabin1), RyR2-bound FKBP12.6 (calstabin2), and RyR3-bound FKBP12 (calstabin1).

[00249] As used herein, "RyR" also includes an "RyR protein" and an "RyR analogue." An "RyR analogue" is a functional variant of the RyR protein, having RyR biological activity, which has 60% or greater amino-acid-sequence homology with the RyR protein. The RyR of the present invention are unphosphorylated, phosphorylated (*e.g.*, by PKA), or hyperphosphorylated (*e.g.*, by PKA). As further used herein, the term "RyR biological activity" refers to the activity of a protein or peptide that demonstrates an ability to associate physically with, or bind with, FKBP12 (calstabin1) in the case of RyR1 and RyR3, and FKBP12.6 (calstabin2) in the case of RyR2 (*i.e.*, binding of approximately two

fold or, approximately five fold, above the background binding of a negative control), under the conditions of the assays described herein.

[00250] As used herein, "FKBP" includes both an "FKBP protein" and an "FKBP analogue," whether it is FKBP12 (calstabin1) or FKBP12.6 (calstabin2). Unless otherwise indicated herein, "protein" shall include a protein, protein domain, polypeptide, or peptide, and any fragment thereof. An "FKBP analogue" is a functional variant of the FKBP protein, having FKBP biological activity, that has 60% or greater amino-acid-sequence homology with the FKBP protein, whether it is FKBP12 (calstabin1) or FKBP12.6 (calstabin2). As further used herein, the term "FKBP biological activity" refers to the activity of a protein or peptide that demonstrates an ability to associate physically with, or bind with, unphosphorylated or non-hyperphosphorylated RyR2 (*i.e.*, binding of approximately two fold, or approximately five fold, above the background binding of a negative control), under the conditions of the assays described herein.

[00251] FKBP binds to the RyR channel, one molecule per RyR subunit. Accordingly, as used herein, the term "RyR-bound FKBP" includes a molecule of an FKBP12 (calstabin1) protein that is bound to an RyR1 protein subunit or a tetramer of FKBP12 that is in association with a tetramer of RyR1, a molecule of FKBP12.6 (calstabin2) protein that is bound to an RyR2 protein subunit or a tetramer of FKBP12.6 that is in association with a tetramer of RyR2, and a molecule of an FKBP12 (calstabin1) protein that is bound to an RyR3 protein subunit or a tetramer of FKBP12 that is in association with a tetramer of RyR3. Therefore, "RyR-bound FKBP" refers to "RyR1-bound FKBP12," "RyR2-bound FKBP12.6," and "RyR3-bound FKBP12."

[00252] In accordance with the method of the present invention, a "decrease" or "disorder" in the level of RyR-bound FKBP in cells of a subject refers to a detectable decrease, diminution or reduction in the level of RyR-bound FKBP in cells of the subject. Such a decrease is limited or prevented in cells of a subject when the decrease is in any way halted, hindered, impeded, obstructed or reduced by the administration of compounds of the invention, such that the level of RyR-bound FKBP in cells of the subject is higher than it would otherwise be in the absence of the administered compound.

[00253] The level of RyR-bound FKBP in a subject is detected by standard assays and techniques, including those readily determined from the known art (*e.g.*, immunological

techniques, hybridization analysis, immunoprecipitation, Western-blot analysis, fluorescence imaging techniques and/or radiation detection, etc.), as well as any assays and detection methods disclosed herein. For example, protein is isolated and purified from cells of a subject using standard methods known in the art, including, without limitation, extraction from the cells (*e.g.*, with a detergent that solubilizes the protein) where necessary, followed by affinity purification on a column, chromatography (*e.g.*, FTLC and HPLC), immunoprecipitation (with an antibody), and precipitation (*e.g.*, with isopropanol and a reagent such as Trizol). Isolation and purification of the protein is followed by electrophoresis (*e.g.*, on an SDS-polyacrylamide gel). A decrease in the level of RyR-bound FKBP in a subject, or the limiting or prevention thereof, can be determined by comparing the amount of RyR-bound FKBP detected prior to the administration of JTV-519 or a compound of the invention (in accordance with methods described below) with the amount detected a suitable time after administration of the compound.

[00254] An increase in the ratio of FKBP bound to RyR in the RyR-FKBP complex in cells of a subject can be achieved, for example, by reducing or preventing PKA-phosphorylation of the RyR receptor, inhibiting dissociation of FKBP and RyR in cells of the subject; by increasing binding between FKBP and RyR in cells of the subject; or by stabilizing the RyR-FKBP complex in cells of a subject. A decrease in the level of RyR-bound FKBP in cells of a subject is limited or prevented, for example, by reducing or preventing PKA-phosphorylation of the RyR receptor, inhibiting dissociation of FKBP and RyR in cells of the subject; by increasing binding between FKBP and RyR in cells of the subject; or by stabilizing the RyR-FKBP complex in cells of a subject.

[00255] As used herein, the term "inhibiting dissociation" includes blocking, decreasing, inhibiting, limiting or preventing the physical dissociation or separation of an FKBP subunit from an RyR molecule in cells of the subject, and blocking, decreasing, inhibiting, limiting or preventing the physical dissociation or separation of an RyR molecule from a FKBP subunit in cells of the subject. As further used herein, the term "increasing binding" includes enhancing, increasing, or improving the ability of phosphorylated RyR to associate physically with FKBP (*e.g.*, binding of approximately two fold or, approximately five fold, above the background binding of a negative control) in cells of the subject and enhancing, increasing or improving the ability of FKBP to associate physically with phosphorylated RyR (*e.g.*, binding of approximately two fold, or, approximately five fold,

above the background binding of a negative control) in cells of the subject. Additionally, a decrease in the level of RyR-bound FKBP in cells of a subject is limited or prevented by directly decreasing the level of phosphorylated RyR in cells of the subject or by indirectly decreasing the level of phosphorylated RyR in the cells (*e.g.*, by targeting an enzyme (such as PKA) or another endogenous molecule that regulates or modulates the functions or levels of phosphorylated RyR in the cells). In one embodiment, the level of phosphorylated RyR in the cells is decreased by at least 10% in the method of the present invention. In another embodiment, the level of phosphorylated RyR is decreased by at least 20%.

[00256] The subject of the present invention are *in vitro* and *in vivo* systems, including, without limitation, isolated or cultured cells or tissues, non-cell *in vitro* assay systems and an animal (*e.g.*, an amphibian, a bird, a fish, a mammal, a marsupial, a human, a domestic animal (such as a cat, dog, monkey, mouse or rat) or a commercial animal (such as a horse, cow or pig).

[00257] The cells of a subject include striated muscle cells. A striated muscle is a muscle in which the repeating units (sarcomeres) of the contractile myofibrils are arranged in registry throughout the cell, resulting in transverse or oblique striations that are observed at the level of a light microscope. Examples of striated muscle cells include, without limitation, voluntary (skeletal) muscle cells and cardiac muscle cells. In one embodiment, the cell used in the method of the present invention is a human cardiac muscle cell. As used herein, the term "cardiac muscle cell" includes cardiac muscle fibers, such as those found in the myocardium of the heart. Cardiac muscle fibers are composed of chains of contiguous heart-muscle cells, or cardiomyocytes, joined end to end at intercalated disks. These disks possess two kinds of cell junctions: expanded desmosomes extending along their transverse portions, and gap junctions, the largest of which lie along their longitudinal portions.

[00258] A decrease in the level of RyR-bound FKBP is limited or prevented in cells of a subject by administering the compounds of the invention to the subject; this would also permit contact between cells of the subject and the compounds of the invention. The compounds of the invention are modulators of calcium-ion channels. In addition to regulating Ca^{2+} levels in myocardial cells, the compounds of the invention modulate the Na^{+} current and the inward-rectifier K^{+} current in cells, such as guinea pig ventricular cells, and inhibits the delayed-rectifier K^{+} current in cells, such as guinea pig atrial cells.

Pharmaceutical and Veterinary Composition

[00259] In one aspect, the compounds of the invention are formulated into pharmaceutical compositions for administration to human and non-human animal subjects in a biologically compatible form suitable for administration *in vivo*. According to another aspect, the present invention provides a pharmaceutical composition comprising compounds of the invention in admixture with a pharmaceutically acceptable diluent and/or carrier. The pharmaceutically-acceptable carrier must be "acceptable" in the sense of being compatible with the other ingredients of the composition and not deleterious to the recipient thereof. The pharmaceutically-acceptable carrier employed herein is selected from various organic or inorganic materials that are used as materials for pharmaceutical formulations and which are incorporated as analgesic agents, buffers, binders, disintegrants, diluents, emulsifiers, excipients, extenders, glidants, solubilizers, stabilizers, suspending agents, tonicity agents, vehicles and viscosity-increasing agents. If necessary, pharmaceutical additives, such as antioxidants, aromatics, colorants, flavor-improving agents, preservatives, and sweeteners, are also added. Examples of acceptable pharmaceutical carriers include carboxymethyl cellulose, crystalline cellulose, glycerin, gum arabic, lactose, magnesium stearate, methyl cellulose, powders, saline, sodium alginate, sucrose, starch, talc and water, among others.

[00260] The pharmaceutical formulations of the present invention are prepared by methods well-known in the pharmaceutical arts. For example, the compounds of the invention are brought into association with a carrier and/or diluent, as a suspension or solution. Optionally, one or more accessory ingredients (*e.g.*, buffers, flavoring agents, surface active agents, and the like) also are added. The choice of carrier is determined by the solubility and chemical nature of the compounds, chosen route of administration and standard pharmaceutical practice.

[00261] The compounds of the invention are administered to a subject by contacting target cells (*e.g.*, cardiac muscle cells) *in vivo* in the subject with the compounds. The compounds of the invention are contacted with (*e.g.*, introduced into) cells of the subject using known techniques utilized for the introduction and administration of proteins, nucleic acids and other drugs. Examples of methods for contacting the cells with (*i.e.*, treating the cells with) the compounds of the invention include, without limitation, absorption, electroporation, immersion, injection, introduction, liposome delivery, transfection, transfusion, vectors and other drug-delivery vehicles and methods. When the target cells

are localized to a particular portion of a subject, it is desirable to introduce the compounds of the invention directly to the cells, by injection or by some other means (*e.g.*, by introducing the compounds into the blood or another body fluid). The target cells are contained in tissue of a subject and are detected by standard detection methods readily determined from the known art, examples of which include, without limitation, immunological techniques (*e.g.*, immunohistochemical staining), fluorescence imaging techniques, and microscopic techniques.

[00262] Additionally, the compounds of the present invention are administered to a human or non-human animal subject by known procedures including, without limitation, oral administration, sublingual or buccal administration, parenteral administration, transdermal administration, via inhalation or intranasally, vaginally, rectally, and intramuscularly. The compounds of the invention are administered parenterally, by epifascial, intracapsular, intracranial, intracutaneous, intrathecal, intramuscular, intraorbital, intraperitoneal, intraspinal, intrasternal, intravascular, intravenous, parenchymatous, subcutaneous or sublingual injection, or by way of catheter. In one embodiment, the agent is administered to the subject by way of delivery to the subject's muscles including, but not limited to, the subject's cardiac muscles. In an embodiment, the agent is administered to the subject by way of targeted delivery to cardiac muscle cells *via* a catheter inserted into the subject's heart.

[00263] In one embodiment, formulations of the compounds of the invention for oral administration are presented as capsules, tablets, powders, granules or as a suspension or solution. The formulation has conventional additives, such as lactose, mannitol, corn starch or potato starch. The formulation also is presented with binders, such as crystalline cellulose, cellulose derivatives, acacia, corn starch or gelatins. Additionally, the formulation is presented with disintegrators, such as corn starch, potato starch or sodium carboxymethylcellulose. The formulation also is presented with dibasic calcium phosphate anhydrous or sodium starch glycolate. Finally, the formulation is presented with lubricants, such as talc or magnesium stearate.

[00264] In another embodiment, the compounds of the invention can be administered orally as nutritional formulations for animals. In one embodiment, the nutritional formulation, which comprises the compounds of the invention, is in the form of an animal feed. In another embodiment, the nutritional formulation, which comprises the compounds

of the invention, is in the form of nutritional treats. In another embodiment, the nutritional formulation, which comprises the compounds of the invention, is in the form of a nutritional supplement. In another embodiment, the nutritional formulation, which comprises the compounds of the invention, is in the form of food pellet. Any one of the nutritional compositions of the invention is palatable to non-human animals. In one embodiment, the nutritional formulation can be a nutritional treat, for example but not limited to a dog biscuit, which comprises typical dog biscuit ingredients such as cereal grains, vegetable or animal proteins, fat and by-products, and a therapeutically active amount of the compounds of the invention, or combination thereof. In another embodiment, the nutritional treats can be nutritional pellets, for example, of a size suitable for ingestion by cats. The nutritional pellets comprise typical cat pellet ingredients such as cereal grains, vegetable, fish or animal proteins, fat and by-products, and a therapeutically active amount of the compounds of the invention, or combination thereof. In another embodiment, wherein the nutritional formulation comprising the compounds of the invention, or combination thereof, is made for horses, the main ingredient may be mostly grass or hay. In another embodiment, the nutritional formulation may be poultry feed which also comprises the compounds of the invention, or combination thereof.

[00265] In another embodiment, a liquid oral formulation for non-human animals comprises the compounds of the invention, or combination thereof, and taste masking agents which render the liquid oral formulation palatable to non-human animals.

[00266] For parenteral administration (*i.e.*, administration by injection through a route other than the alimentary canal), the compounds of the invention are combined with a sterile aqueous solution that is isotonic with the blood of the subject. Such a formulation is prepared by dissolving a solid active ingredient in water containing physiologically-compatible substances, such as sodium chloride, glycine and the like, and having a buffered pH compatible with physiological conditions, so as to produce an aqueous solution, then rendering said solution sterile. The formulation is presented in unit or multi-dose containers, such as sealed ampoules or vials. The formulation is delivered by any mode of injection, including, without limitation, epifascial, intracapsular, intracranial, intracutaneous, intrathecal, intramuscular, intraorbital, intraperitoneal, intraspinal, intrasternal, intravascular, intravenous, parenchymatous, subcutaneous, or sublingual or by way of catheter into the subject's heart.

[00267] For transdermal administration, the compounds of the invention are combined with skin penetration enhancers, such as propylene glycol, polyethylene glycol, isopropanol, ethanol, oleic acid, *N*-methylpyrrolidone and the like, which increase the permeability of the skin to the compounds of the invention and permit the compounds to penetrate through the skin and into the bloodstream. The compounds of the invention/enhancer composition also are further combined with a polymeric substance, such as ethylcellulose, hydroxypropyl cellulose, ethylene/vinylacetate, polyvinyl pyrrolidone, and the like, to provide the composition in gel form, which are dissolved in a solvent, such as methylene chloride, evaporated to the desired viscosity and then applied to backing material to provide a patch.

[00268] In some embodiments, the composition is in unit dose form such as a tablet, capsule or single-dose vial. Suitable unit doses, *i.e.*, therapeutically effective amounts, can be determined during clinical trials designed appropriately for each of the conditions for which administration of a chosen compound is indicated and will, of course, vary depending on the desired clinical endpoint. The present invention also provides articles of manufacture for treating and preventing disorders, such as cardiac disorders, in a subject. The articles of manufacture comprise a pharmaceutical composition of one or more of the compounds of the invention as described herein. The articles of manufacture are packaged with indications for various disorders that the pharmaceutical compositions are capable of treating and/or preventing. For example, the articles of manufacture comprise a unit dose of a compound disclosed herein that is capable of treating or preventing a muscular disorder, and an indication that the unit dose is capable of treating or preventing a certain disorder, for example an arrhythmia.

[00269] In accordance with a method of the present invention, the compounds of the invention are administered to the subject (or are contacted with cells of the subject) in an amount effective to limit or prevent a decrease in the level of RyR-bound FKBP in the subject, particularly in cells of the subject. This amount is readily determined by the skilled artisan, based upon known procedures, including analysis of titration curves established *in vivo* and methods and assays disclosed herein. A suitable amount of the compounds of the invention effective to limit or prevent a decrease in the level of RyR-bound FKBP in the subject ranges from about 5 mg/kg/day to about 20 mg/kg/day, and/or is an amount sufficient to achieve plasma levels ranging from about 300 ng/ml to about 1000 ng/ml. In

an embodiment, the amount of compounds of the invention ranges from about 10 mg/kg/day to about 20 mg/kg/day.

Uses

[00270] The present invention provides a new range of therapeutic treatments for subjects with various disorders involving modulation of the RyR receptors, particularly skeletal muscular disorders (RyR1), cardiac (RyR2) disorders, and cognitive (RyR3) disorders.

[00271] In one embodiment of the present invention, the subject has not yet developed a disorder, such as cardiac disorders (*e.g.*, exercise-induced cardiac arrhythmia). In another embodiment of the present invention, the subject is in need of treatment for a disorder, including a cardiac disorder.

[00272] The compounds of the invention treat or prevent various disorders, which include, but are not limited to, cardiac disorders and diseases, skeletal muscular disorders and diseases, cognitive disorders and diseases, malignant hyperthermia, diabetes, and sudden infant death syndrome. Cardiac disorder and diseases include, but are not limited to, irregular heartbeat disorders and diseases; exercise-induced irregular heartbeat disorders and diseases; sudden cardiac death; exercise-induced sudden cardiac death; congestive heart failure; chronic obstructive pulmonary disease; and high blood pressure. Irregular heartbeat disorders and diseases include exercise-induced irregular heartbeat disorders and diseases that include, but are not limited to, atrial and ventricular arrhythmia; atrial and ventricular fibrillation; atrial and ventricular tachyarrhythmia; atrial and ventricular tachycardia; catecholaminergic polymorphic ventricular tachycardia (CPVT), arrhythmogenic right ventricular cardiomyopathy (ARVC), and exercise-induced variants thereof. Skeletal muscular disorder and diseases include, but are not limited to, skeletal muscle fatigue, exercise-induced skeletal muscle fatigue, muscular dystrophy, bladder disorders, and incontinence. Cognitive disorders and diseases include, but are not limited to, Alzheimer's Disease, forms of memory loss, and age-dependent memory loss. One skilled in the art will recognize still other diseases, including but not limited to muscular and cardiac disorders, that the compounds of the invention are be useful to treat, in accordance with the information provided herein.

[00273] The amount of compounds of the invention effective to limit or prevent a decrease in the level of RyR2-bound FKBP12.6 in the subject is an amount effective to prevent cardiac arrhythmogenic condition in the subject, including non-human animal. Cardiac arrhythmia is a disturbance of the electrical activity of the heart that manifests as an abnormality in heart rate or heart rhythm. As used herein, an amount of compounds of the invention "effective to prevent cardiac arrhythmogenic condition" includes an amount of compounds of the invention effective to prevent the development of the clinical impairment or symptoms of cardiac arrhythmia (*e.g.*, palpitations, fainting, ventricular fibrillation, ventricular tachycardia and sudden cardiac death). The amount of compounds of the invention which is effective to prevent cardiac arrhythmia in a subject will vary depending upon the particular factors of each case, including the type of exercise-induced cardiac arrhythmia, the subject's weight, the severity of the subject's condition, and the mode of administration of the compounds of the invention. This amount is readily determined by the skilled artisan, based upon known procedures, including clinical trials, and the methods disclosed herein. In some embodiments, the amount of the compounds of the invention effective to prevent the exercise-induced cardiac arrhythmia is an amount effective to prevent exercise-induced sudden cardiac death in the subject. In some other embodiments, the compounds of the invention prevent exercise-induced cardiac arrhythmia and exercise-induced sudden cardiac death in the subject.

[00274] Because of its ability to stabilize RyR-bound FKBP and maintain and restore balance in the context of dynamic PKA phosphorylation and dephosphorylation of RyR, the compounds of the invention are also useful in treating a subject who has already experienced clinical symptoms of these various disorders. For example, if the symptoms of the disorder are observed in the subject early enough, the compounds of the invention are effective in limiting or preventing a further decrease in the level of RyR-bound FKBP in the subject.

[00275] The subject of the present invention can be a candidate for exercise-induced cardiac disorders, such as exercise-induced cardiac arrhythmia. Exercise-induced cardiac arrhythmia is a heart condition (*e.g.*, a ventricular fibrillation or ventricular tachycardia, including any that leads to sudden cardiac death) that develops during/after a subject has undergone physical exercise. A "candidate" for an exercise-induced cardiac disorder is a subject who is known to be, or is believed to be, or is suspected of being, at risk for

developing a cardiac disorder during/after physical exercise. Examples of candidates for exercise-induced cardiac arrhythmia include, without limitation, an animal/person known to have catecholaminergic polymorphic ventricular tachycardia (CPVT); an animal/person suspected of having CPVT; and an animal/person who is known to be, or is believed to be, or is suspected of being at risk for developing cardiac arrhythmia during/after physical exercise, and who is about to exercise, is currently exercising or has just completed exercise. As discussed above, CPVT is an inherited disorder in individuals with structurally-normal hearts. It is characterized by stress-induced ventricular tachycardia – a lethal arrhythmia that causes sudden cardiac death. In subjects with CPVT, physical exertion and/or stress induce bidirectional and/or polymorphic ventricular tachycardias that lead to sudden cardiac death (SCD) in the absence of detectable structural heart disease. Individuals with CPVT have ventricular arrhythmias when subjected to exercise, but do not develop arrhythmias at rest.

[00276] Accordingly, in still another embodiment of the present invention, the subject has been exercising, or is currently exercising, and has developed an exercise-induced disorder. In this case, the amount of the compounds of the invention effective to limit or prevent a decrease in the level of RyR-bound FKBP in the subject is an amount of compound effective to treat the exercise-induced disorder in the subject. As used herein, an amount of compounds of the invention "effective to treat an exercise-induced disorder" includes an amount of compound of the invention effective to alleviate or ameliorate the clinical impairment or symptoms of the exercise-induced disorder (*e.g.*, in the case of cardiac arrhythmia, palpitations, fainting, ventricular fibrillation, ventricular tachycardia, and sudden cardiac death). The amount of compounds of the invention effective to treat an exercise-induced disorder in a subject will vary depending upon the particular factors of each case, including the type of exercise-induced disorder, the subject's weight, the severity of the subject's condition, and the mode of administration of the compounds of the invention. This amount is readily determined by the skilled artisan, based upon known procedures, including clinical trials, and methods disclosed herein. Thus, in some embodiments, the compounds of the invention treat exercise-induced disorders in the subject.

[00277] The subject of the present invention can be a candidate with a cardiac arrhythmogenic condition which includes but is not limited to heart failure, sudden cardiac

death, cardiac arrhythmias, e.g., ventricular and atrial tachycardia; atrial arrhythmia, including atrial tachyarrhythmia and atrial fibrillation (both sustained and non-sustained); ventricular arrhythmia, including ventricular tachyarrhythmia, ventricular fibrillation; and stress or exercise-induced cardiac arrhythmia, catecholaminergic polymorphic ventricular tachycardia (CPVT), arrhythmogenic right ventricular cardiomyopathy (ARVD/C), hypertrophic cardiomyopathy, dilated cardiomyopathy, restrictive cardiomyopathy, sick sinus syndrome, atrial standstill sinus tachycardia, and/or stress- or exercise-induced sudden cardiac death.

[00278] In one aspect, the present invention provides a method for treating a cardiac arrhythmogenic condition which includes but is not limited to heart failure, sudden cardiac death, cardiac arrhythmias, e.g., ventricular and atrial tachycardia; atrial arrhythmia, including atrial tachyarrhythmia and atrial fibrillation (both sustained and non-sustained); ventricular arrhythmia, including ventricular tachyarrhythmia, ventricular fibrillation; and stress or exercise-induced cardiac arrhythmia, catecholaminergic polymorphic ventricular tachycardia (CPVT), arrhythmogenic right ventricular cardiomyopathy (ARVD/C), hypertrophic cardiomyopathy, dilated cardiomyopathy, restrictive cardiomyopathy, sick sinus syndrome, atrial standstill sinus tachycardia, and/or stress- or exercise-induced sudden cardiac death. The present invention further provides a method for treating exercise-induced disorders in a subject. The present invention also provides a method for treating arrhythmogenic right ventricular cardiomyopathy (ARVD/C). The method comprises administering the compounds of the invention to the subject in an amount effective to treat ARVD/C in the subject. A suitable amount of the compounds of the invention effective to treat, for example, ARVD/C in the subject ranges from about 5 mg/kg/day to about 20 mg/kg/day, and/or is an amount sufficient to achieve plasma levels ranging from about 300 ng/ml to about 1000 ng/ml. The present invention also provides a method for preventing an exercise-induced disorder in a subject. The method comprises administering the compounds of the invention to the subject in an amount effective to prevent the exercise-induced disorder in the subject. A suitable amount of the compounds of the invention effective to prevent the exercise-induced disorder in the subject ranges from about 5 mg/kg/day to about 20 mg/kg/day, and/or is an amount sufficient to achieve plasma levels ranging from about 300 ng/ml to about 1000 ng/ml. Additionally, the present invention provides a method for preventing exercise-induced disorders in a subject. The method comprises administering the compounds of the invention to the subject in an amount

effective to prevent a cardiac arrhythmogenic condition, including an exercise-induced disorder in the subject. A suitable amount of the compounds of the invention effective to prevent an exercise-induced disorder in the subject ranges from about 5 mg/kg/day to about 20 mg/kg/day, and/or is an amount sufficient to achieve plasma levels ranging from about 300 ng/ml to about 1000 ng/ml.

[00279] Additionally, the compounds of the invention prevent irregular heartbeat disorders in subjects with heterozygous defects in the calstabin2/FKBP12.6 gene, or reduced amount of calstabin2/FKBP12.6 mRNA or calstabin2.FKBP12.6 protein in cardiac tissue.

[00280] The compounds of the invention can be used alone, in combination with each other, or in combination with other agents that have cardiovascular activity including, but not limited to, diuretics, anticoagulants, antiplatelet agents, antiarrhythmics, inotropic agents, chronotropic agents, α and β blockers, angiotensin inhibitors and vasodilators. Further, such combinations of the compounds of the present invention and other cardiovascular agents are administered separately or in conjunction. In addition, the administration of one element of the combination is prior to, concurrent to or subsequent to the administration of other agent(s).

[00281] In various embodiments of the above-described methods, the exercise-induced cardiac arrhythmia in the subject is associated with VT. In some embodiments, the VT is CPVT. In other embodiments of these methods, the subject is a candidate for exercise-induced cardiac arrhythmia, including candidates for exercise-induced sudden cardiac death.

[00282] In view of the foregoing methods, the present invention also provides use of the compounds of the invention in a method for limiting or preventing a decrease in the level of RyR-bound FKBP in a subject who is a candidate for a disorder. The present invention also provides use of the compounds of the invention in a method for treating or preventing a muscular disorder in a subject. Furthermore, the present invention provides use of the compounds of the invention in a method for treating or preventing exercise-induced muscular disorders in a subject.

[00283] Accordingly, the present invention further provides a method for assaying the effects of the compounds of the invention in preventing disorders and diseases

associated with the RyR receptors. The method comprises the steps of: (a) obtaining or generating a culture of cells containing RyR; (b) contacting the cells with one or more of the compounds of the invention; (c) exposing the cells to one or more conditions known to increase phosphorylation of RyR in cells; and (d) determining if the one or more compounds from the invention limits or prevents a decrease in the level of RyR-bound FKBP in the cells. As used herein, a cell "containing RyR" is a cell in which RyR, including RyR1, RyR2, and RyR3, or a derivative or homologue thereof, is naturally expressed or naturally occurs. Conditions known to increase phosphorylation of RyR in cells include, without limitation, PKA. In one embodiment, the culture of cells containing RyR can be a cardiac myocyte cell which has reduced levels of calstabin2 mRNA, and/or calstabin2 protein.

[00284] In the method of the present invention, cells are contacted with one or more of the compounds of the invention by any of the standard methods of effecting contact between drugs/agents and cells, including any modes of introduction and administration described herein. The level of RyR-bound FKBP in the cell is measured or detected by known procedures, including any of the methods, molecular procedures and assays known to one of skill in the art or described herein. In one embodiment of the present invention, the one or more compounds of the invention limits or prevents a decrease in the level of RyR-bound FKBP in the cells.

[00285] In another aspect, the invention provides a method for determining the effect of a test compound on an arrhythmogenic condition in a subject, including non-human animal such as canine, wherein the method comprises the steps of: administering a placebo compound to a non-human animal, wherein the animal is characterized by decreased calstabin2 levels in cardiac tissue, and administering a test compound to a second non-human animal, wherein the second animal is characterized by decreased calstabin2 levels in cardiac tissue, and determining the rate of electrical signals that control the heartbeat rhythm in the presence of the placebo and the test compound, wherein a test compound that improves irregular heartbeat rhythm in the second animal compared with the first animal, is indicative of a test compound that prevents or treats cardiac arrhythmias, and wherein the non-human animal is not a mouse. Alternatively, the test compound and the placebo are administered, at different times, to the same non-human animal, which is characterized by a

decreased calstabin2 levels in cardiac tissue. In one embodiment, determining the rate of electrical signals that control the heartbeat rhythm is measured by electrocardiogram.

[00286] RyR, including RyR1, RyR2, and RyR3, has been implicated in a number of biological events in cells. For example, it has been shown that RyR2 channels play an important role in EC coupling and contractility in cardiac muscle cells. Therefore, it is clear that preventive drugs designed to limit or prevent a decrease in the level of RyR-bound FKBP in cells, particularly RyR2-bound FKBP12.6 in cardiac muscle cells, are useful in the regulation of a number of RyR-associated biological events, including EC coupling and contractility. Thus, the one or more compounds of the invention are evaluated for effect on EC coupling and contractility in cells, particularly cardiac muscle cells, and therefore, usefulness for preventing exercise-induced sudden cardiac death.

[00287] Accordingly, the method of the present invention further comprises the steps of contacting one or more compounds of the invention with a culture of cells containing RyR; and determining if the one or more compounds has an effect on an RyR-associated biological event in the cells. As used herein, an "RyR-associated biological event" includes a biochemical or physiological process in which RyR levels or activity have been implicated. As disclosed herein, examples of RyR-associated biological events include, without limitation, EC coupling and contractility in cardiac muscle cells. According to this method of the present invention, the one or more compounds of the invention are contacted with one or more cells (such as cardiac muscle cells) *in vitro*. For example, a culture of the cells is incubated with a preparation containing the one or more compounds of the invention. The compounds' effect on a RyR-associated biological event then is assessed by any biological assays or methods known in the art, including immunoblotting, single-channel recordings and any others disclosed herein.

[00288] The present invention is further directed to one or more compounds of the invention identified by the above-described identification method, as well as a pharmaceutical composition comprising the compound and a pharmaceutically acceptable carrier and/or diluent. The compounds are useful for preventing exercise-induced sudden cardiac death in a subject, and for treating or preventing other RyR-associated conditions. As used herein, an "RyR-associated condition" is a condition, disease, or disorder in which RyR level or activity has been implicated, and includes an RyR-associated biological event. The RyR-associated condition is treated or prevented in the subject by administering to the

subject an amount of the compound effective to treat or prevent the RyR-associated condition in the subject. This amount is readily determined by one skilled in the art. In one embodiment, the present invention provides a method for preventing exercise-induced sudden cardiac death in a subject, by administering the one or more compounds of the invention to the subject in an amount effective to prevent the exercise-induced sudden cardiac death in the subject.

[00289] The present invention also provides an *in vivo* method for assaying the effectiveness of the compounds of the invention in preventing disorders and diseases associated with the RyR receptors. The method comprises the steps of: (a) obtaining or generating an animal containing RyR; (b) administering one or more of the compounds of the invention to the animal; (c) exposing the animal to one or more conditions known to increase phosphorylation of RyR in cells; and (d) determining the extent the compound limits or prevents a decrease in the level of RyR-bound FKBP in the animal. The method further comprises the steps of: (e) administering one or more of the compounds of the invention to an animal containing RyR; and (f) determining the extent of the effect of the compound on a RyR-associated biological event in the animal. Also provided is a pharmaceutical composition comprising this compound; and a method for preventing exercise-induced sudden cardiac death in a subject, by administering this compound to the subject in an amount effective to prevent the exercise-induced sudden cardiac death in the subject.

[00290] It has been demonstrated that compounds which block PKA activation would be expected to reduce the activation of the RyR channel, resulting in less release of calcium into the cell. Compounds that bind to the RyR channel at the FKBP binding site, but do not come off the channel when the channel is phosphorylated by PKA, would also be expected to decrease the activity of the channel in response to PKA activation or other triggers that activate the RyR channel. Such compounds would also result in less calcium release into the cell.

[00291] By way of example, the diagnostic assays screen for the release of calcium into cells *via* the RyR channel, using calcium-sensitive fluorescent dyes (*e.g.*, Fluo-3, Fura-2, and the like). Cells are loaded with the fluorescent dye of choice, then stimulated with RyR activators to determine the reduction of the calcium-dependent fluorescent signal (Brillantes, *et al.*, Stabilization of calcium release channel (ryanodine receptor) function by

FK506-binding protein. *Cell*, 77:513-23, 1994; Gillo, *et al.*, Calcium entry during induced differentiation in murine erythroleukemia cells. *Blood*, 81:783-92, 1993; Jayaraman, *et al.*, Regulation of the inositol 1,4,5-trisphosphate receptor by tyrosine phosphorylation. *Science*, 272:1492-94, 1996). Calcium-dependent fluorescent signals are monitored with a photomultiplier tube, and analyzed with appropriate software. This assay can easily be automated to screen the compounds of the invention using multiwell dishes.

[00292] To demonstrate that the compounds of the invention inhibit the PKA-dependent activation of RyR-mediated intracellular calcium release, an assay involves the expression of recombinant RyR channels in a heterologous expression system, such as Sf9, HEK293, or CHO cells. RyR could also be co-expressed with beta-adrenergic receptors. This would permit assessment of the effect of compounds of the invention on RyR activation, in response to addition of beta-adrenergic receptor agonists.

[00293] The level of PKA phosphorylation of RyR2 which correlates with the degree of heart failure also is assayed and then used to determine the efficacy of the one or more compounds of the invention to block the PKA phosphorylation of the RyR2 channel. Such an assay is based on the use of antibodies that are specific for the RyR2 protein. For example, the RyR2-channel protein is immunoprecipitated and then back-phosphorylated with PKA and [γ - ^{32}P]-ATP. The amount of radioactive [^{32}P] label that is transferred to the RyR2 protein then is measured using a phosphorimager (Marx, *et al.*, PKA phosphorylation dissociates FKBP12.6 from the calcium release channel (ryanodine receptor): defective regulation in failing hearts. *Cell*, 101:365-76, 2000).

[00294] Another assay of the compounds of the invention involves use of a phosphoepitope-specific antibody that detects RyR1 that is PKA phosphorylated on Ser 2843 or RyR2 that is PKA phosphorylated on Ser 2809. Immunoblotting with such an antibody can be used to assess efficacy of these compounds for therapy for heart failure and cardiac arrhythmias. Additionally, RyR2 S2809A and RyR2 S2809D knock-in mice are used to assess efficacy of therapy for heart failure and cardiac arrhythmias. Such mice further provide evidence that PKA hyperphosphorylation of RyR2 is a contributing factor in heart failure and cardiac arrhythmias by showing that the RyR2 S2809A mutation inhibits heart failure and arrhythmias, and that the RyR2 S2809D mutation worsens heart failure and arrhythmias.

[00295] Therefore, in a specific embodiment, the present invention provides a method of treating heart failure, atrial fibrillation or exercise-induced cardiac arrhythmia, comprising administering to an animal in need thereof, a therapeutically effective amount of a compound selected from the compounds of the invention.

[00296] Intracellular Ca^{2+} leak is proposed as a principal mediator of depressed muscle performance and dystrophic muscle remodeling. Muscular dystrophies are heterogeneous hereditary diseases characterized by weakness and progressive muscle wasting. Of all forms of muscular dystrophies involving the dystrophin-associated protein complex (referred to as dystrophinopathies), Duchenne muscular dystrophy (DMD) is one of the most frequent genetic diseases (X-linked; 1 in 3,500 boys) with death usually occurring before age 30 by respiratory and/or cardiac failure in high numbers of patients. Becker muscular dystrophy (BMD) represents a milder form of the disease associated with a reduction in the amount or expression of a truncated form of the dystrophin protein whereas Duchenne patients have been characterized by complete absence or very low levels of dystrophin. Duchenne and Becker's muscular dystrophy (DMD/BMD) are caused by mutations in the gene encoding the 427-kDa cytoskeletal protein dystrophin. However, with increasing age in BMD cardiac symptoms are more common than in DMD patients and do not correlate with skeletal muscle symptoms. Since genetic screening will not eliminate DMD due to a high incidence of sporadic cases, an effective therapy is highly desirable. DMD/BMD have been consistently associated with disturbed intracellular calcium metabolism. Because alterations of intracellular Ca^{2+} concentrations in DMD myofibers are believed to represent a central pathogenic mechanism, development of a therapeutic intervention that prevents intracellular Ca^{2+} abnormalities as a cause of skeletal muscle degeneration is highly desirable.

[00297] It is well established that lack of dystrophin expression is the primary genetic defect in DMD and BMD. However, the key mechanism leading to progressive muscle damage is largely unknown. It has been suggested that elevations of intracellular Ca^{2+} concentrations ($[\text{Ca}^{2+}]_i$) under resting conditions directly contributed to toxic muscle cell (myofiber) damage and concurrent activation of Ca^{2+} -dependent proteases. Since calpain activity is increased in necrotic muscle fibers of mdx mice and calpain dysfunction contributes to limb-girdle muscular dystrophy, preventing activation of calcium-dependent proteases by inhibiting intracellular Ca^{2+} elevations represents a strategy to prevent muscle

wasting in DMD. Significant differences in $[Ca^{2+}]_i$ between normal and dystrophic muscles have been reported in myotubes and animal models including the dystrophin-deficient mdx mouse. Intracellular Ca^{2+} elevations are prevented by administration of a pharmaceutical composition comprising a compound of the invention.

[00298] The present invention also provides a method of diagnosis of a disease or disorder in a subject, said method comprising: obtaining a cell or tissue sample from the subject; obtaining DNA from the cell or tissue; comparing the DNA from the cell or tissue with a control DNA encoding RyR to determine whether a mutation is present in the DNA from the cell or tissue, the presence of a mutation indicating a disease or disorder. In one embodiment, the mutation is a RyR2 mutation on chromosome 1q42-q43. In another embodiment, the mutation is one or more CPTV mutations. In another embodiment, the mutation may be a mutation which is present in the DNA encoding RyR2 of a SIDS subject. The diagnostic method is used to detect the presence of a disease or disorder in an adult, a child or a fetus. The disease and disorders include, but are not limited to, cardiac disorders and diseases, skeletal muscular disorders and diseases, cognitive disorders and diseases, malignant hyperthermia, diabetes, and sudden infant death syndrome. Cardiac disorder and diseases include, but are not limited to, irregular heartbeat disorders and diseases; exercise-induced irregular heartbeat disorders and diseases; sudden cardiac death; exercise-induced sudden cardiac death; congestive heart failure; chronic obstructive pulmonary disease; and high blood pressure. Irregular heartbeat disorders and diseases include exercise-induced irregular heartbeat disorders and diseases that include, but are not limited to, atrial and ventricular arrhythmia; atrial and ventricular fibrillation; atrial and ventricular tachyarrhythmia; atrial and ventricular tachycardia; catecholaminergic polymorphic ventricular tachycardia (CPVT); and exercise-induced variants thereof. Skeletal muscular disorder and diseases include, but are not limited to, skeletal muscle fatigue, exercise-induced skeletal muscle fatigue, muscular dystrophy, bladder disorders, and incontinence. Cognitive disorders and diseases include, but are not limited to, Alzheimer's Disease, forms of memory loss, and age-dependent memory loss.

[00299] The present invention further provides a method of diagnosis of disorders and diseases in a subject, said method comprising: obtaining cells or tissue sample from the subject; incubating the cells or tissue sample with the compound of the invention under conditions which increase phosphorylation of RyR in cells; determining (a) whether RyR

bound to calstabin (i.e. RyR1 bound to calstabin1, RyR2 bound to calstabin2, or RyR3 bound to calstabin1) is increased in the cells or tissue compared to RyR bound to calstabin in control cells or tissues said control cells or tissues lacking mutant RyR calcium channels, or (b) whether a decrease in release of calcium occurs in RyR channels compared to a lack of decrease in release of calcium in the control cells; an increase in RyR-bound calstabin in (a) indicating a disorder or disease in the subject or a decrease in release of calcium in RyR channels in (b) compared to the control cells indicating a cardiac disease or disorder in the subject. The diagnostic method is used to detect the presence of a disease or disorder in an adult, a child or a fetus. The disease and disorders include, but are not limited to, cardiac disorders and diseases, skeletal muscular disorders and diseases, cognitive disorders and diseases, malignant hyperthermia, diabetes, and sudden infant death syndrome. Cardiac disorder and diseases include, but are not limited to, irregular heartbeat disorders and diseases; exercise-induced irregular heartbeat disorders and diseases; sudden cardiac death; exercise-induced sudden cardiac death; congestive heart failure; chronic obstructive pulmonary disease; and high blood pressure. Irregular heartbeat disorders and diseases include exercise-induced irregular heartbeat disorders and diseases that include, but are not limited to, atrial and ventricular arrhythmia; atrial and ventricular fibrillation; atrial and ventricular tachyarrhythmia; atrial and ventricular tachycardia; catecholaminergic polymorphic ventricular tachycardia (CPVT); arrhythmogenic right ventricular cardiomyopathy and exercise-induced variants thereof. Skeletal muscular disorder and diseases include, but are not limited to, skeletal muscle fatigue, exercise-induced skeletal muscle fatigue, muscular dystrophy, bladder disorders, and incontinence. Cognitive disorders and diseases include, but are not limited to, Alzheimer's Disease, forms of memory loss, and age-dependent memory loss.

[00300] The present invention further provides a method of diagnosis of a cardiac disorder or disease in a subject, said method comprising: obtaining cardiac cells or a tissue sample from the subject; incubating the cardiac cells or tissue sample with the compound of the invention under conditions which increase phosphorylation of RyR2 in cells; determining (a) whether RyR2 bound to calstabin2 is increased in the cells or tissue compared to RyR2 bound to calstabin2 in control cells or tissues said control cells or tissues lacking mutant RyR2 calcium channels, or (b) whether a decrease in release of calcium occurs in RyR2 channels compared to a lack of decrease in release of calcium in the control cells; an increase in RyR2-bound calstabin2 in (a) indicating a disorder or disease in the

subject or a decrease in release of calcium in RyR2 channels in (b) compared to the control cells indicating a cardiac disease or disorder in the subject. The provided method is used to diagnose CPTV. The provided method also is used to diagnose sudden infant death syndrome (SIDS). The provided method additionally is used to diagnose cardiac irregular heartbeat disorders and diseases; exercise-induced irregular heartbeat disorders and diseases; sudden cardiac death; exercise-induced sudden cardiac death; congestive heart failure; chronic obstructive pulmonary disease; and high blood pressure. Irregular heartbeat disorders and diseases include exercise-induced irregular heartbeat disorders and diseases that include, but are not limited to, atrial and ventricular arrhythmia; atrial and ventricular fibrillation; atrial and ventricular tachyarrhythmia; atrial and ventricular tachycardia; catecholaminergic polymorphic ventricular tachycardia (CPVT); arrhythmogenic right ventricular cardiomyopathy and exercise-induced variants thereof.

[00301] The invention can be further described with the following non-limiting examples.

[00302] Canine Subject Population and Tissue Collection—Left ventricular tissue samples were obtained at post-mortem from 4 Boxer dogs with ARVC, 3 Doberman pinschers with dilated cardiomyopathy, 3 Beagle dogs with experimental heart failure secondary to rapid ventricular pacing, and 3 healthy controls. Boxer and Doberman pinscher dogs were electively euthanized at the request of their owners due to progressive heart disease. Sections of the left ventricular free wall (1 cm³) were snap-frozen in liquid nitrogen directly following euthanasia. Tissues from healthy control dogs and dogs with experimentally induced heart failure were also procured directly after euthanasia. Samples were stored at -70°C until processed. Antemortem diagnosis of ARVC was made based on presence of ventricular tachyarrhythmias with left-bundle branch block morphology (>1,000 ventricular premature complexes/24h), and if present, syncope, myocardial dysfunction, and congestive heart failure. A 3-channel ambulatory ECG recording system (Delmar Medical Systems, Irvine, CA) was used as previously described. (Baumwart, RD, et al, Clinical, echocardiographic, and electrocardiographic abnormalities in Boxers with cardiomyopathy and left ventricular systolic dysfunction: 48 cases (1985-2003). J Am. Vet Med Assoc 2005;226:1102-1104). Postmortem histopathology on formalin-fixed tissue blocks of ventricular tissue was performed on Boxer dogs to confirm histological changes such as fatty infiltration of the ventricular myocardium, which is consistent with ARVC.

(Smucker,ML, Naturally occurring cardiomyopathy in the Doberman pinscher: a possible large animal model of human cardiomyopathy? J Am Coll Cardiol 1990;16:200-206). Antemortem diagnosis of dilated cardiomyopathy in Doberman pinscher dogs was made based on myocardial systolic dysfunction, presence of ventricular arrhythmias, and radiographic evidence of congestive heart failure. Dogs with pacing-induced heart failure underwent between 50-80 days of rapid ventricular pacing (180-240bpm) and exhibited clinical and radiographic evidence of congestive heart failure and echocardiographic evidence of systolic dysfunction. Myocardial function was determined using transthoracic echocardiography with a GE Vivid 7 or GE System V echocardiographic system and calculation of left ventricular fractional shortening (GE Medical Systems, Waukesha, WI). Control dogs included adult Beagle dogs free of cardiac disease.

[00303] Oligonucleotide Microarray Analysis—mRNA levels of *calstabin2* was determined using a canine-specific oligonucleotide array by methods known in the art. (Oyama,MA, Chittur,S. Genomic expression patterns of cardiac tissues from dogs with dilated cardiomyopathy. Am J Vet Res 2005;66:1140-1155). Individual oligonucleotide arrays were performed on each tissue sample. Differential expression of *calstabin2* between affected and control tissue was determined by comparing intensities of probe signals using a 2-tailed Student *t* test (significance designated at values of $P < 0.05$ with Benjamini-Hochberg false discovery correction). Results were reported as the relative fold-change in *calstabin2* expression. Thus, a negative fold-change indicated lesser expression in Boxers as compared to another experimental group. To validate the findings of the microarray-based results, differential gene expression for *calstabin2* was confirmed by use of real time quantitative PCR. The primer pair (Forward-AGGGACTTGAGCCAGTTACCTTT (referred to as SEQ ID NO:3), Reverse-AATTCTGTTGGCTGCTGACTTACA (referred to as SEQ ID NO:4)] was selected by use of commercially available software and synthesized by use of a nucleic acid synthesis and purification system in accordance with manufacturer's specifications. All reactions were performed in triplicate. Reactions that did not contain template RNA were included as negative-control samples.

[00304] Western Blot Analysis and Immunoprecipitation—RyR2 was immunoprecipitated from samples by incubating 250 µg of heart homogenate with anti-RyR antibody (2 µl 5029 Ab) in 0.5 ml of a modified RIPA buffer (50 mM Tris-HCl (pH 7.4), 0.9% NaCl, 5.0 mM NaF, 1.0 mM Na₃VO₄, 1.0% Triton-X100, and protease inhibitors) for

1 hr at 4°C. The samples were subsequently incubated with Protein A sepharose beads (Amersham Pharmacia Biotech, Piscataway, NJ) at 4°C for 1 hour, after which, the beads were washed three times with RIPA buffer. Samples were heated to 95°C and size fractionated on 6% SDS PAGE to detect RyR2 and 15% SDS-PAGE to detect calstabin2. Immunoblots were developed using anti-RyR (5029, 1:5000 dilution in 5% milk TBS-T), PKA phospho-specific Ab (P2809, 1:10,000 dilution) or anti-FKBP Ab (1:2000 dilution) as previously described.

[00305] DNA sequencing—DNA samples from 10 Boxer dogs previously diagnosed with ARVC were evaluated. DNA samples were compared to two unaffected Labrador retriever dogs as well as the published canine (Boxer dog) genome sequence. Genomic DNA samples were prepared from whole blood samples by methods known in the art. (Meurs, KM, Nine polymorphisms within the head and hinge region of the feline cardiac beta-myosin heavy chain gene. *Anim Genet* 2000;31:231). In short, cells were osmotically lysed in 2X sucrose-Triton, Tris-NH₄Cl buffer and nuclei were pelleted by centrifugation at 800g for 20 min at 4°C. Pellets were resuspended in Saline-EDTA with 1% SDS and 50 µg/ml proteinase K, and incubated overnight at 56°C. The samples were subjected to two successive phenol:chloroform:isoamyl (25:24:1, pH 8) and one chloroform extraction. Finally, the DNA was ethanol precipitated, washed with 75% ethanol, and resuspended in 250 µl of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8). Polymerase chain reaction (PCR) amplification primers were designed for all exons using Primer 3 software and the canine nucleotide sequence information for calstabin published on the Ensemble database (ENSCAFG00000003954).

[00306] PCR amplification-- Standard PCR amplifications were carried out using NH₄SO₄ amplification buffer, 0.1 units/µl reaction volume Taq DNA polymerase (Fermentas, Hanover, MD), 2.5 mM MgCl₂, 12.5 µM each dNTP, 2.5 mM of each PCR amplification primer and 100 ng of template DNA. Samples were denatured for 5 min at 94°C followed by 40 cycles of 94°C for 20 seconds; 58°C for 30 seconds, 72°C for 30 seconds; and finally 72°C for 7 minutes. The annealing temperature was optimized to accommodate the respective primer requirement.

[00307] Residual amplification primers and dNTPs were removed from the PCR product using ExoSapIt enzymatic treatment (GE Healthcare Bio-Sciences Corp., Piscataway, NJ). Amplicons were then subjected to nucleotide sequence determination and

analyzed on an ABI Prism 377 Sequencer (Applied Biosystems, Foster City, CA) using a forward and reverse primer for each reaction for every sample.

[00308] The sequences were compared for nucleotide sequence changes between affected dogs, the published canine sequence (derived from a Boxer dog) and the controls. Base pair changes were considered to be causative for ARVC if they met the following criteria: were present in all of the affected dogs, changed a conserved amino acid and changed the amino acid to one of a different polarity, acid/base status or structure.

[00309] Antemortem and Postmortem Diagnosis—Affected Boxer dogs demonstrated ventricular arrhythmias with left-bundle branch block morphology (Figure 8) and significant replacement of cardiac myocytes with adipose tissue (Figure 9). These findings are typical of ARVC in this breed. Boxer dog arrhythmogenic cardiomyopathy is a disease of both the left and right ventricular myocardium but histopathologic lesions tend to be concentrated in the right ventricle. To compare Boxer dog arrhythmogenic cardiomyopathy with other forms of myocardial disease that predominantly affect the left ventricle, left ventricular tissues was used in this study.

Example 1

[00310] Oligonucleotide Microarray Analysis—Calstabin2 mRNA levels were significantly lower in Boxer dog hearts with ARVC as compared to healthy controls, Doberman pinschers with dilated cardiomyopathy, and dogs with pacing-induced heart failure (fold change in Boxer dogs; -13.3 vs. control, $P<0.05$; -7.0 vs. Doberman pinschers, $P<0.05$; -3.5 vs. rapid ventricular pacing, $P<0.05$). Microarray data were validated using real time RT-qPCR, which indicated a -12.5 fold-change in Boxer dogs vs. controls ($P<0.05$).

[00311] These results demonstrate decreased myocardial calstabin2 message and protein, and specifically, reduced calstabin2 associated with the RyR2 complex in the hearts of Boxer dogs with arrhythmogenic cardiomyopathy. Calstabin2 depletion in the RyR2 complex has been demonstrated in human heart failure, and dogs with pacing-induced heart failure revealed significantly decreased calstabin2 protein in the RyR2 complex as compared to control. (Yano et al., Circulation 2000;102:2131-2136). PKA phosphorylation of RyR2 phosphorylation, an index of heart failure-associated adrenergic stimulation that

decreases the affinity of calstabin2 for RyR2, was not significantly different between Boxer dogs and controls.

[00312] DNA sequencing—No differences were observed within the promoter, exonic and splice site regions of the calstabin gene between the affected boxer and the controls or published Boxer sequence. This study used non-Boxer controls to avoid the inadvertent inclusion of affected subclinical individuals because significant breed differences involving important cardiac proteins were unlikely. DNA samples were compared to two Labrador retriever dogs since the published canine genome was developed from an adult Boxer dog that may not have been evaluated for ARVC.

Example 2

[00314] Calstabin2 Deficiency in Myocardial Tissue and in the RyR2 macromolecular complex—Immunoblotting of left ventricular (LV) homogenates revealed significantly decreased calstabin2 protein in the hearts of affected Boxers vs. control (Figure 1A). Using co-immunoprecipitation, it was found that a significant reduction in the amount of calstabin2 bound to the RyR2 complex in the hearts of affected Boxers as compared to controls (Figure 1B). Relative amounts of calstabin2 and RyR2 were calculated using densitometry of gels and indicated significantly reduced calstabin2 in the RyR2 complex in the hearts of affected Boxer dogs vs. control (calstabin2 per RyR2 complex: Boxer dogs, 0.51 ± 0.04 vs. control, 3.81 ± 0.22 ; $P < 0.0001$; Figure 1B). PKA phosphorylation of RyR2 was not significantly different between Boxer dogs and controls (Figure 1B).

[00315] The findings of calstabin2 depletion in the RyR2 complex in the hearts of Boxer dogs with arrhythmogenic cardiomyopathy help characterize this condition as a useful model of calstabin2 deficiency and may provide new insights into the etiology and pathophysiology of certain forms of arrhythmogenic cardiomyopathies in humans.

Hypothetical Examples

Example 3

[00316] Active compounds of the invention, for example S4, S7, S20, S36, or a combination thereof are expected to repair Ca^{2+} leak in RyR2-calstabin2 complex in Sarcoplasmic Reticulum (SR) of failing hearts from Boxer dogs with ARVC. SR vesicles can be isolated from subject canines, which are boxer dogs diagnosed with ARVC, and

controls, dogs known not to have ARVC. SR vesicles from subjects and controls can be incubated under conditions that allow Ca^{2+} uptake. Ca^{2+} can be monitored spectrophotometrically with a Ca^{2+} sensitive dye such as Fluo3 (Molecular Probes). When the Ca^{2+} uptake reaches a plateau, various concentrations of FK506 can be added in the presence of thapsigargin to inhibit SR Ca^{2+} -ATP activity and the resulting Ca^{2+} leak can be monitored in the presence or absence of the compounds of the invention.

Example 4

[00317] Compounds of the invention are expected to increase the ratio of calstabin2 to Ryr2 in the RyR2-calstabin2 complex. Canines suspected of having a cardiac condition, for example ARVC, can be diagnosed by measurement of the levels of calstabin2 (FKBP12.6) in cardiac tissue (see Example 1 and 2). In addition to the measurement of calstabin2 (FKBP12.6) levels, ARVC diagnosis of canines suspected of having a cardiac condition can be confirmed by optional monitoring of the canine's heart rhythm through ECG, Holter monitoring or another suitable technique that monitors heart rate and rhythm. Subject canines, diagnosed with ARVC, are animals which demonstrate decreased level of calstabin2 in cardiac tissue. Subject canines may also demonstrate depolarization-repolarization and conduction abnormalities as detected by ECG.

[00318] Subject canines can be treated with an active compound of the invention, for example S4, S7, S20, S36, or a combination thereof by daily administration of a dog biscuit which comprises an active compound in a therapeutic amount. It is expected that an active compound can restore binding of calstabin2 (FKBP12.6) to RyR2 in the RyR2-calstabin2 complex.

Example 5

[00319] Canine Model of ARVC: Subject animals are canines which are diagnosed with ARVC by a method described in Example 1 or Example 2. Animals can be anesthetized with pentobarbital (30 mg/kg), and their hearts can be removed. Atrial and ventricular tissue can be dissected, immediately flash-frozen in liquid nitrogen, and stored at -80.degree. C.

[00320] Immunoprecipitation and Back-Phosphorylation of RyR2 : Cardiac membranes (100 μg), can be prepared from left atrial (LA) or right ventricle (RV) tissue as previously described (Marx, et al., PKA Phosphorylation Dissociates FKBP12.6 from the

Calcium Release Channel (Ryanodine Receptor): Defective Regulation in Failing Hearts, Cell, 101:365-376, 2000) can be suspended in 0.5 ml of RIPA buffer (50 mM Tris-HCL [pH 7.4], 0.9% NaCl, 0.25% TritonX-100, 5 mM NaF, and protease inhibitors), and can be incubated with rabbit anti-RyR2 antibody overnight at 4°C. Protein A sepharose beads can be added, and allowed to incubate at 4°C for 1 h. Protein A beads can be subsequently washed with 1X kinase buffer (50 mM Tris-HCL, 50 mM piperazine-N,N'-bis[2-ethanesulfonic acid], 8 mM MgCl₂, and 10 mM EGTA [pH 6.8]), and then can be resuspended in 1.5X kinase buffer. The reaction can be initiated with PKA (5 units), 100 μM MgATP, and [γ-³²P] ATP (NEN Life Sciences, Boston); can be allowed to incubate for 8 min at room temperature, and is then can be stopped with 5μl of 6X loading buffer (4% SDS and 0.25 M DTT). Samples can be heated to 95°C, and then can be size-fractionated on 6% SDS-PAGE. RyR2 radioactivity is quantified using a Molecular Dynamics Phosphoimager, and Imagequant software (Amersham Pharmacia Biotech, Piscataway, N.J.). Values can be divided by the amount of immunoprecipitated RyR2 (determined by immunoblotting and densitometry), and expressed as the inverse of the [γ-³²P] ATP signal.

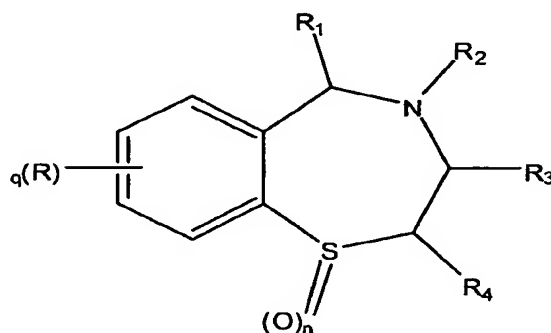
[00321] Calstabin2 (FKBP12.6) Rebinding with Compounds of the Invention: RyR2 can be immunoprecipitated from ventricular SR (100 μg), and washed with kinase buffer. The immunoprecipitated RyR2 can be phosphorylated with PKA (5 units) and 100 μM MgATP at room temperature, and the reaction can be terminated after 8 min by washing with ice-cold RIPA buffer. Recombinant calstabin2 (FKBP12.6; 200 nM) can be subsequently incubated with the phosphorylated RyR2 at room temperature, in the presence or absence of various concentrations of compounds of the invention (for example 1 μM concentration). The proteins can be size-fractionated by 15% SDS PAGE, and immunoblotted for calstabin2 (FKBP12.6). Binding of calstabin2 to RyR2 precipitated from ventricular SR isolated from canines with ARVC can be measured in the presence or absence of the compounds of the invention.

[00322] Calstabin2 (FKBP12.6) Rebinding in Presence of Compounds of the Invention : Any physiologic significance of FKBP-12.6 rebinding in the presence of compounds of the invention can be demonstrated by RyR2 single-channel measurements in planar lipid bilayers.

What is claimed is:

1. A method for detecting an arrhythmogenic condition in a subject non-human animal, comprising:
 - (1) measuring a level of calstabin2 (FKBP12.6) in a cardiac tissue of a subject non-human animal, and
 - (2) comparing the level of calstabin2 (FKBP12.6) in the cardiac tissue of the subject non-human animal to a level of calstabin2 (FKBP12.6) in a cardiac tissue of a control non-human animal, the control non-human animal known not to have an arrhythmogenic condition,wherein a reduced level of calstabin2 (FKBP12.6) in the cardiac tissue of the subject non-human animal compared to the level of calstabin2 (FKBP12.6) in the cardiac tissue of the control non-human animal indicates the presence of an arrhythmogenic condition.
2. The method of claim 1, wherein the calstabin2 is present in an RyR2-calstabin2 complex.
3. The method of claim 1, wherein the non-human animal is selected from the group consisting of canines, felines, equines, porcine, poultry, ruminants, and rodents.
4. The method of claim 3, wherein the canine is selected from a breed of Boxer, German Shepherd, Miniature Schnauzer, West Highland White terrier, Dachshund, English Springer Spaniel, Golden Retriever, Doberman Pinscher, Newfoundland, Cocker Spaniel, Grate Dane, Irish Wolfhound, Afghan Hound, and Saluki.
5. The method of claim 1 or 2, wherein the arrhythmogenic condition is an arrhythmogenic right ventricular cardiomyopathy (ARVC).
6. The method of claim 5, wherein the animal is a canine.
7. The method of claim 5, wherein the reduced level of calstabin2 (FKBP12.6) in the cardiac tissue of the subject non-human animal that indicates the presence of arrhythmogenic right ventricular cardiomyopathy is reduced by about 2 times to about a level which is below the threshold of detection compared to the level of calstabin2 (FKBP12.6) in the cardiac tissue of the control non-human animal.

8. A method for treating a disorder or disease in a non-human animal, the method comprising administering to the non-human animal an agent known to affect the interaction of the calstabin protein to a RyR receptor in a RyR-calstabin protein complex.
9. The method of claim 8, wherein the agent is selected from the group of compounds of the general Formula I:



wherein,

n is 0, 1, or 2;

q is 0, 1, 2, 3, or 4;

each R is independently selected from the group consisting of H, halogen, -OH, -NH₂, -NO₂, -CN, -CF₃, -OCF₃, -N₃, -SO₃H, -S(=O)₂alkyl, -S(=O)alkyl, -OS(=O)₂CF₃, acyl, -O-acyl, alkyl, alkoxyl, alkylamino, alkylaryl amino, alkylthio, cycloalkyl, alkylaryl, aryl, heteroaryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino; wherein each acyl, -O-acyl, alkyl, alkoxyl, alkylamino, alkylaryl amino, alkylthio, cycloalkyl, alkylaryl, aryl, heteroaryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino may be optionally substituted;

R₁ is selected from the group consisting of H, oxo, alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl; wherein each alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl may be optionally substituted;

R₂ is selected from the group consisting of H, -C(=O)R₅, -C(=S)R₆, -SO₂R₇, -P(=O)R₈R₉, -(CH₂)_m-R₁₀, alkyl, aryl, alkylaryl, heteroaryl, cycloalkyl, cycloalkylalkyl, and heterocyclyl; wherein each alkyl, aryl, alkylaryl, heteroaryl, cycloalkyl, cycloalkylalkyl, and heterocyclyl may be optionally substituted;

R_3 is selected from the group consisting of H, $-\text{CO}_2\text{Y}$, $-\text{C}(=\text{O})\text{NHY}$, acyl, $-\text{O}$ -acyl, alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl; wherein each acyl, alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl may be optionally substituted; and wherein Y is selected from the group consisting of H, alkyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl, and wherein each alkyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl may be optionally substituted;

R_4 is selected from the group consisting of H, alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl; wherein each alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl may be optionally substituted;

R_5 is selected from the group consisting of $-\text{NR}_{15}\text{R}_{16}$, $-(\text{CH}_2)_q\text{NR}_{15}\text{R}_{16}$, $-\text{NHN}\text{R}_{15}\text{R}_{16}$, $-\text{NHOH}$, $-\text{OR}_{15}$, $-\text{C}(=\text{O})\text{NHN}\text{R}_{15}\text{R}_{16}$, $-\text{CO}_2\text{R}_{15}$, $-\text{C}(=\text{O})\text{NR}_{15}\text{R}_{16}$, $-\text{CH}_2\text{X}$, acyl, alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl may be optionally substituted, and wherein q is 1, 2, 3, 4, 5, or 6;

R_6 is selected from the group consisting of $-\text{OR}_{15}$, $-\text{NHN}\text{R}_{15}\text{R}_{16}$, $-\text{NHOH}$, $-\text{NR}_{15}\text{R}_{16}$, $-\text{CH}_2\text{X}$, acyl, alkenyl, alkyl, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkenyl, alkyl, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl may be optionally substituted;

R_7 is selected from the group consisting of $-\text{OR}_{15}$, $-\text{NR}_{15}\text{R}_{16}$, $-\text{NHN}\text{R}_{15}\text{R}_{16}$, $-\text{NHOH}$, $-\text{CH}_2\text{X}$, alkyl, alkenyl, alkynyl, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl; wherein each alkyl, alkenyl, alkynyl, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl may be optionally substituted;

R_8 and R_9 independently are selected from the group consisting of OH, acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl may be optionally substituted;

R_{10} is selected from the group consisting of $-NR_{15}R_{16}$, OH, $-SO_2R_{11}$, $-NHSO_2R_{11}$, $C(=O)(R_{12})$, $NHC(=O)(R_{12})$, $-OC(=O)(R_{12})$, and $-P(=O)R_{13}R_{14}$;

R_{11} , R_{12} , R_{13} , and R_{14} independently are selected from the group consisting of H, OH, NH_2 , $-NHNH_2$, $-NHOH$, acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl may be optionally substituted;

X is selected from the group consisting of halogen, $-CN$, $-CO_2R_{15}$, $-C(=O)NR_{15}R_{16}$, $-NR_{15}R_{16}$, $-OR_{15}$, $-SO_2R_7$, and $-P(=O)R_8R_9$; and

R_{15} and R_{16} independently are selected from the group consisting of H, acyl, alkenyl, alkoxyl, OH, NH_2 , alkyl, alkylamino, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl may be optionally substituted; and optionally R_{15} and R_{16} together with the N to which they are bonded may form a heterocycle which may be substituted;

the nitrogen in the benzothiazepine ring may optionally be a quaternary nitrogen; and

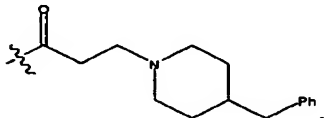
enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, and prodrugs thereof;

provided that when q is 0 and n is 0, then R_2 is not H, Et, $-C(=O)NH_2$, $(=O)NHPh$, $-C(=S)NH-nButyl$, $-C(=O)NHC(=O)CH_2Cl$, $-C(=O)H$, $-C(=O)Me$, $-C(=O)Et$, $-C(=O)CH=CH_2$, $-S(=O)_2Me$, or $-S(=O)_2Et$;

further provided that when q is 0 and n is 1 or 2, then R_2 is not $-C(=O)Me$, $-C(=O)Et$, $-S(=O)_2Me$, or $-S(=O)_2Et$;

further provided that when q is 1, and R is Me, Cl, or F at the 6 position of the benzothiazepene ring, then R_2 is not H, Me, $-C(=O)H$, $-C(=O)Me$, $-C(=O)Et$, $-C(=O)Ph$, $-S(=O)_2Me$, or $-S(=O)_2Et$; and

further provided that when q is 1, n is 0, and R is OCT₃, OH, C₁-C₃ alkoxyl at the 7 position of the benzothiazepene ring, then R₂ is not H, -C(=O)CH=CH₂, or

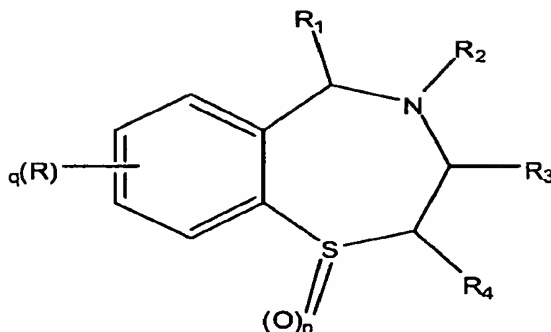


10. The method of claim 8, wherein the agent is selected from the group consisting of the compounds: S1, S2, S3, S4, S5, S6, S7, S9, S11, S12, S13, S14, S19, S20, S22, S23, S25, S26, S36, S37, S38, S40, S43, S44, S45, S46, S47, S48, S49, S50, S51, S52, S53, S54, S55, S56, S57, S58, S59, S60, S61, S62, S63, S64, S66, S67, S68, S69, S70, S71, S72, S73, S74, S75, S76, S77, S78, S79, S80, S81, S82, S83, S84, S85, S86, S87, S88, S89, S90, S91, S92, S93, S94, S95, S96, S97, S98, S99, S100, S101, S102, S103, S104, S105, S107, S108, S109, S110, S111, S112, S113, S114, S115, S116, S117, S118, S119, S120, S121, S122, and S123, and salts, hydrates, solvates, complexes, and prodrugs thereof.
11. The method of claim 10, wherein the agent is S36 or salts, hydrates, solvates, complexes or prodrugs thereof.
12. The method of claim 10, wherein the agent is S64 or salts, hydrates, solvates, complexes or prodrugs thereof.
13. The method of claim 10, wherein the agent is selected from the group consisting of: S47, S50, S64, S74, S75, S77, S101, S102 and S103 and salts, hydrates, solvates, complexes and prodrugs thereof.
14. The method of claim 8, wherein the disorder or disease is an arrhythmogenic condition, which is selected from the group consisting of: sudden cardiac death, arrhythmogenic right ventricular cardiomyopathy (ARVC), hypertrophic cardiomyopathy, dilated cardiomyopathy, restrictive cardiomyopathy, ventricular arrhythmias, sick sinus syndrome, atrial standstill, atrial fibrillation, sinus tachycardia, and ventricular fibrillations.
15. The method of claim 8, wherein the non-human animal is selected from the group consisting of canines, felines, equines, porcine, poultry, ruminants, and rodents.

16. The method of claim 15, wherein the canine is selected from a breed of Boxer, German Shepherd, Miniature Schnauzer, West Highland White terrier, Dachshund, English Springer Spaniel, Golden Retriever, Doberman Pinscher, Newfoundland, Cocker Spaniel, Grate Dane, Irish Wolfhound, Afghan Hound, and Saluki.
17. The method of claim 8, wherein the agent prevents or inhibits dissociation of the calstabin protein from the RyR receptor.
18. The method of claim 8, wherein the agent increases binding of the calstabin protein to the RyR receptor.
19. The method of claim 8, wherein the agent increases the ratio of calstabin protein to RyR receptor in the RyR receptor-calstabin complex.
20. The method of any one of claims 8 to 19, wherein the RyR receptor is an RyR2 receptor, and the calstabin protein is a calstabin 2 (FKBP12.6) protein.
21. The method of claim 8, wherein the non-human animal demonstrates a reduced level of calstabin2 in a cardiac tissue compared to a level of calstabin2 in a cardiac tissue of a control non-human animal known not to have the cardiac disorder or disease.
22. The method of claim 14, wherein the arrhythmogenic condition is arrhythmogenic right ventricular cardiomyopathy (ARVC).
23. The method of claim 14, wherein the arrhythmogenic condition is sudden cardiac death.
24. The method of claim 22, wherein the non-human animal demonstrates a reduced level of calstabin2 in a cardiac tissue compared to a level of calstabin2 in a cardiac tissue of a control non-human animal known not to have arrhythmogenic right ventricular cardiomyopathy.
25. The method of claim 8 or 24, wherein calstabin2 is present in an RyR2-calstabin2 complex.
26. The method of claim 8, wherein administering the agent prevents or reduces the occurrence or severity of ventricular arrhythmias.

27. The method of claim 8, wherein administering the agent reduces the occurrence of sudden cardiac death.
28. A method for determining the effect of a test compound on an arrhythmogenic condition in a subject, the method comprising the steps of:
- (1) administering a placebo compound to a non-human animal, wherein the animal is characterized by decreased calstabin2 levels in a cardiac tissue, and
 - (2) administering a test compound to a second non-human animal, wherein the second animal is characterized by decreased calstabin2 levels in a cardiac tissue, and
 - (3) determining the rate of electrical signals that control the heartbeat rhythm in the presence of the placebo and the test compound,
- wherein a test compound that improves an irregular heartbeat rhythm in the second animal compared with the first animal is indicative of a test compound that prevents or treats cardiac arrhythmia, and wherein the non-human animal is not a mouse.
29. The method of claim 28, wherein determining the rate of electrical signals that control the heartbeat rhythm is measured by electrocardiogram.
30. A method for determining whether a test compound affects the interaction between calstabin2 and RyR2 in a cardiac myocyte cell characterized by reduced levels of calstabin2, the method comprising:
- (1) contacting a cardiac myocyte cell which has reduced levels of calstabin2 with a test compound,
 - (2) determining the levels of calstabin2 in an RyR2-calstabin2 complex in the cardiac myocyte cell,
- wherein increased level of calstabin2 in the RyR2-calstabin2 complex, after the cardiac myocyte cell has been contacted with the test agent, compared to the calstabin2 levels in an RyR2-calstabin2 complex in a cardiac myocyte cell which has not been contacted with the test compound, is indicative of an agent which affects the interaction between calstabin2 and RyR2.

31. An animal feed comprising an agent from the group of compounds of the general Formula I:



wherein,

n is 0, 1, or 2;

q is 0, 1, 2, 3, or 4;

each R is independently selected from the group consisting of H, halogen, -OH, -NH₂, -NO₂, -CN, -CF₃, -OCF₃, -N₃, -SO₃H, -S(=O)₂alkyl, -S(=O)alkyl, -OS(=O)₂CF₃, acyl, -O-acyl, alkyl, alkoxyl, alkylamino, alkylarylamino, alkylthio, cycloalkyl, alkylaryl, aryl, heteroaryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino; wherein each acyl, -O-acyl, alkyl, alkoxyl, alkylamino, alkylarylamino, alkylthio, cycloalkyl, alkylaryl, aryl, heteroaryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino may be optionally substituted;

R_1 is selected from the group consisting of H, oxo, alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl; wherein each alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl may be optionally substituted;

R_2 is selected from the group consisting of H, -C(=O)R₅, -C(=S)R₆, -SO₂R₇, -P(=O)R₈R₉, -(CH₂)_m-R₁₀, alkyl, aryl, alkylaryl, heteroaryl, cycloalkyl, cycloalkylalkyl, and heterocyclyl; wherein each alkyl, aryl, alkylaryl, heteroaryl, cycloalkyl, cycloalkylalkyl, and heterocyclyl may be optionally substituted;

R_3 is selected from the group consisting of H, -CO₂Y, -C(=O)NHY, acyl, -O-acyl, alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl; wherein each acyl, alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl may be

optionally substituted; and wherein Y is selected from the group consisting of H, alkyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl, and wherein each alkyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl may be optionally substituted;

R₄ is selected from the group consisting of H, alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl; wherein each alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl may be optionally substituted;

R₅ is selected from the group consisting of -NR₁₅R₁₆, -(CH₂)_qNR₁₅R₁₆, -NHNR₁₅R₁₆, -NHOH, -OR₁₅, -C(=O)NHNR₁₅R₁₆, -CO₂R₁₅, -C(=O)NR₁₅R₁₆, -CH₂X, acyl, alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl may be optionally substituted, and wherein q is 1, 2, 3, 4, 5, or 6;

R₆ is selected from the group consisting of -OR₁₅, -NHNR₁₅R₁₆, -NHOH, -NR₁₅R₁₆, -CH₂X, acyl, alkenyl, alkyl, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkenyl, alkyl, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl may be optionally substituted;

R₇ is selected from the group consisting of -OR₁₅, -NR₁₅R₁₆, -NHNR₁₅R₁₆, -NHOH, -CH₂X, alkyl, alkenyl, alkynyl, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl; wherein each alkyl, alkenyl, alkynyl, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl may be optionally substituted;

R₈ and R₉ independently are selected from the group consisting of OH, acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl may be optionally substituted;

R₁₀ is selected from the group consisting of -NR₁₅R₁₆, OH, -SO₂R₁₁, -NHSO₂R₁₁, C(=O)(R₁₂), NHC=O(R₁₂), -OC=O(R₁₂), and -P(=O)R₁₃R₁₄;

R₁₁, R₁₂, R₁₃, and R₁₄ independently are selected from the group consisting of H, OH, NH₂, -NHNH₂, -NHOH, acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl,

alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl may be optionally substituted;

X is selected from the group consisting of halogen, -CN, -CO₂R₁₅, -C(=O)NR₁₅R₁₆, -NR₁₅R₁₆, -OR₁₅, -SO₂R₇, and -P(=O)R₈R₉; and

R₁₅ and R₁₆ independently are selected from the group consisting of H, acyl, alkenyl, alkoxyl, OH, NH₂, alkyl, alkylamino, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl may be optionally substituted; and optionally R₁₅ and R₁₆ together with the N to which they are bonded may form a heterocycle which may be substituted;

the nitrogen in the benzothiazepine ring may optionally be a quaternary nitrogen; and

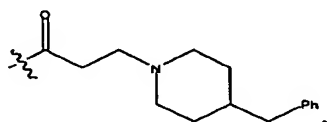
enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, and prodrugs thereof;

provided that when q is 0 and n is 0, then R₂ is not H, Et, -C(=O)NH₂, (=O)NHPh, -C(=S)NH-nButyl, -C(=O)NHC(=O)CH₂Cl, -C(=O)H, -C(=O)Me, -C(=O)Et, -C(=O)CH=CH₂, -S(=O)₂Me, or -S(=O)₂Et;

further provided that when q is 0 and n is 1 or 2, then R₂ is not -C(=O)Me, -C(=O)Et, -S(=O)₂Me, or -S(=O)₂Et;

further provided that when q is 1, and R is Me, Cl, or F at the 6 position of the benzothiazepene ring, then R₂ is not H, Me, -C(=O)H, -C(=O)Me, -C(=O)Et, -C(=O)Ph, -S(=O)₂Me, or -S(=O)₂Et; and

further provided that when q is 1, n is 0, and R is OCT₃, OH, C₁-C₃ alkoxyl at the 7 position of the benzothiazepene ring, then R₂ is not H, -C(=O)CH=CH₂, or



32. The animal feed of claim 31, wherein the agent is selected from the group consisting of the compounds: S1, S2, S3, S4, S5, S6, S7, S9, S11, S12, S13, S14, S19, S20, S22, S23, S25, S26, S36, S37, S38, S40, S43, S44, S45, S46, S47, S48, S49, S50, S51, S52, S53, S54, S55, S56, S57, S58, S59, S60, S61, S62, S63, S64, S66, S67, S68, S69, S70, S71, S72, S73, S74, S75, S76, S77, S78, S79, S80, S81, S82, S83, S84, S85, S86, S87, S88, S89, S90, S91, S92, S93, S94, S95, S96, S97, S98, S99, S100, S101, S102, S103, S104, S105, S107, S108, S109, S110, S111, S112, S113, S114, S115, S116, S117, S118, S119, S120, S121, S122, and S123, and salts, hydrates, solvates, complexes, and prodrugs thereof.
33. The animal feed of claim 31, wherein the agent is S36 or salts, hydrates, solvates, complexes or prodrugs thereof.
34. The animal feed of claim 31, wherein the agent is S64 or salts, hydrates, solvates, complexes or prodrugs thereof.
35. The animal feed of claim 31, wherein the agent is selected from the group consisting of: S47, S50, S64, S74, S75, S77, S101, S102 and S103 S and salts, hydrates, solvates, complexes and prodrugs thereof.

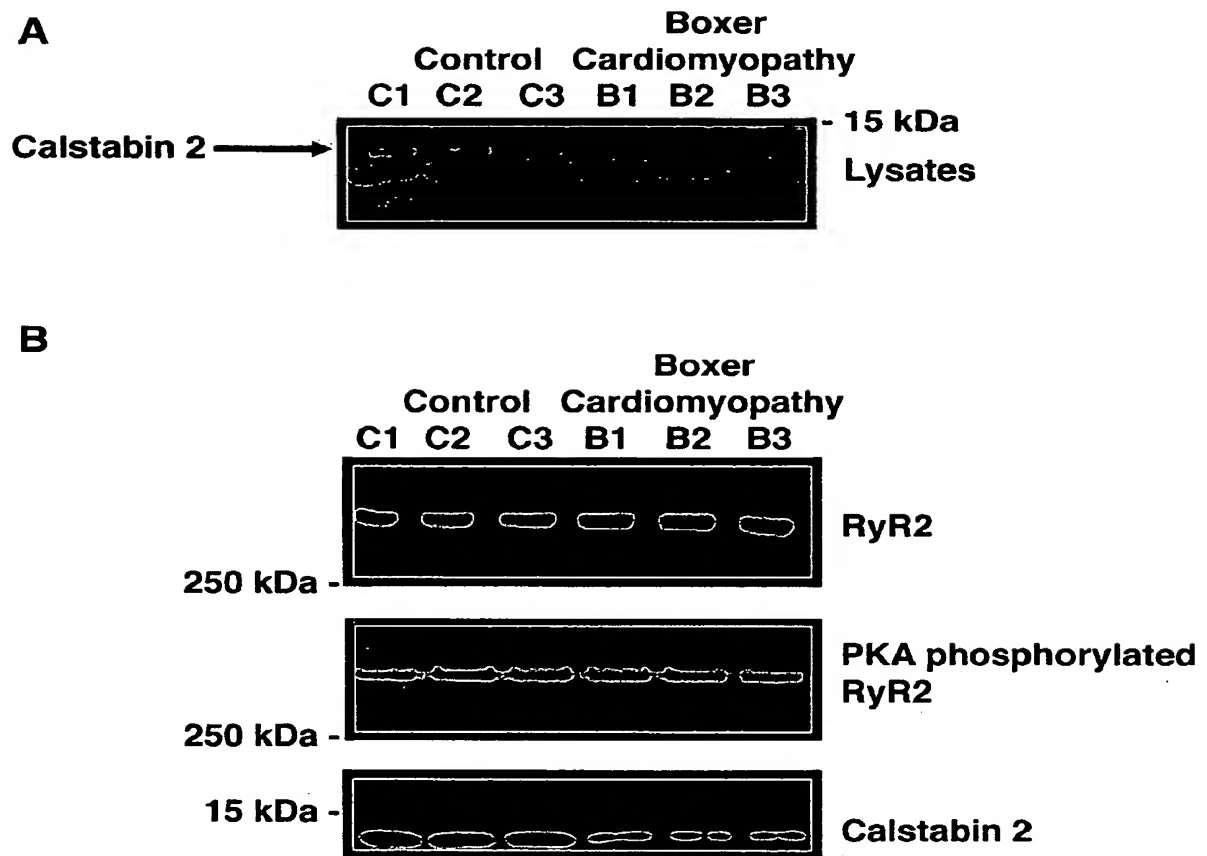


FIG. 1

ATGGGCGTGGAGATCGAGACCATCTCCCCGGGAGACGGAAGGACATTCCCCA
AGAAGGGACAGACGTGTGTGGTGC ACTACACAGGAATGCTCCAAAATGGGAA
GAAATTTGATTCATCCAGAGACAGAAACAAACCTTTCAAGTTCAGAATTGGCA
AACAGGAAGTCATCAAGGGTTTTGAAGAGGGTGCAGCCCAGATGAGCTTGGG
GCAGAGGGGCGAAGCTGACCTGCACCCCCGATGTGGCGTATGGAGCCACGGGC
CACCTGGTGT CATCCCTCCCAATGCCACCCTCATCTTTGACGTGGAGCTGCTC
AACCTAGAGTGA (SEQ ID NO:1)

FIG. 2A

MGVEIETISPGDGRTPPKGGQTCVVHYTGMLQNGKKFDSSRDRNKPFKFRIGKQE
VIKGFEEGAAQMSLGQRAKLTCTPDVAYGATGHPGVIPP NATLIFDV ELLNLE*
(SEQ ID NO:2)

FIG. 2B

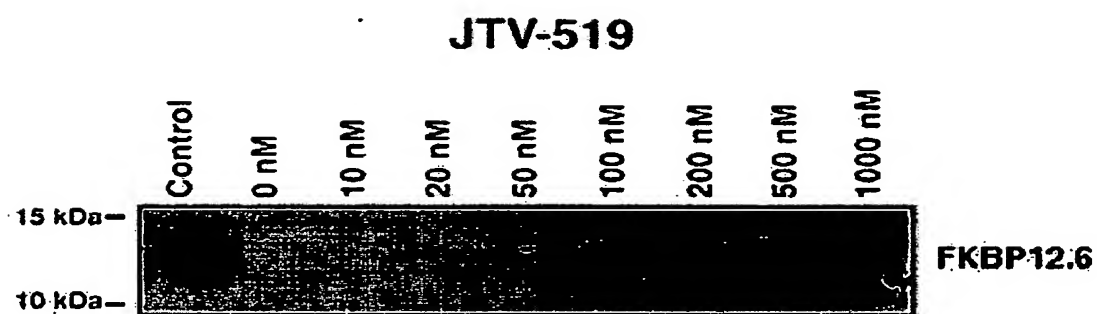


FIG. 3A

Rycal 0.5 nanomolar



FIG. 3B

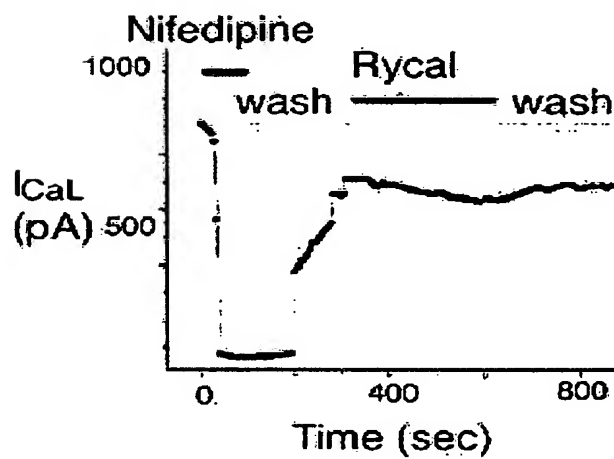


FIG. 3C

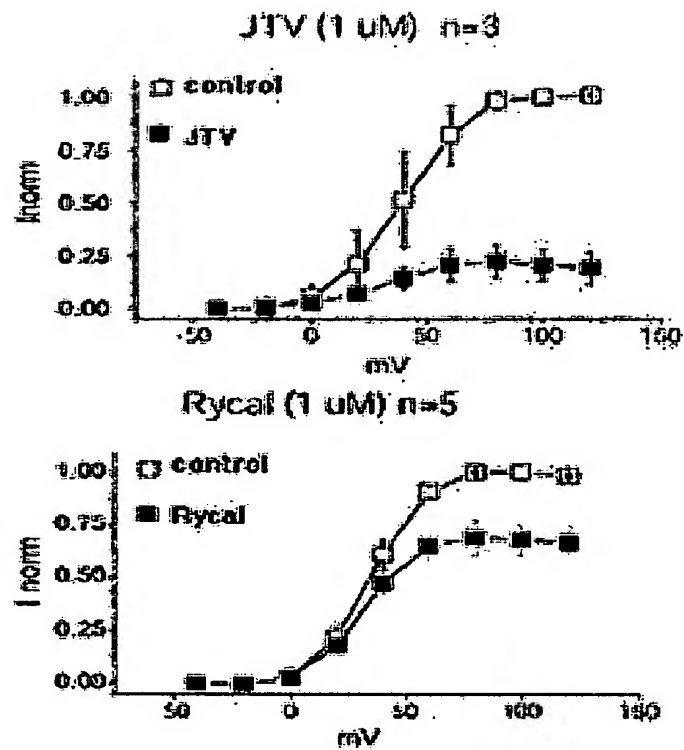


FIG. 3D

ECG at rest

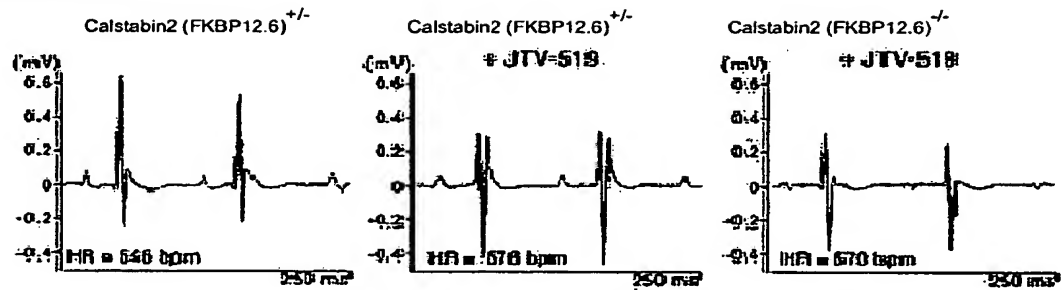


FIG. 4A

ECG following exercise and epinephrine

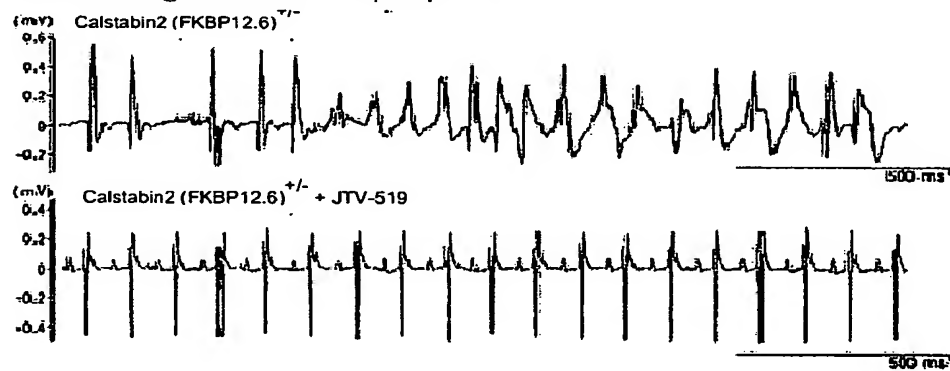


FIG. 4B

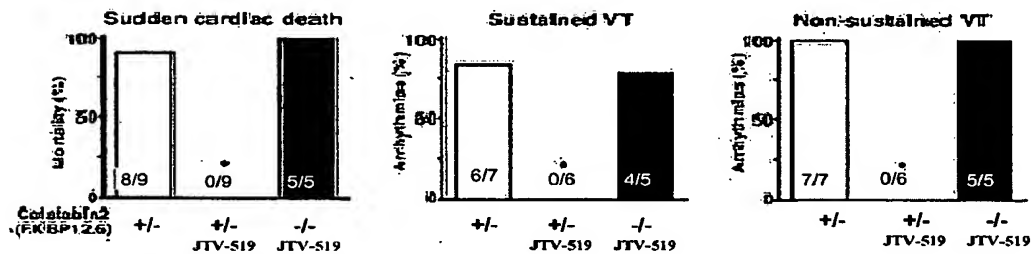


FIG. 4C

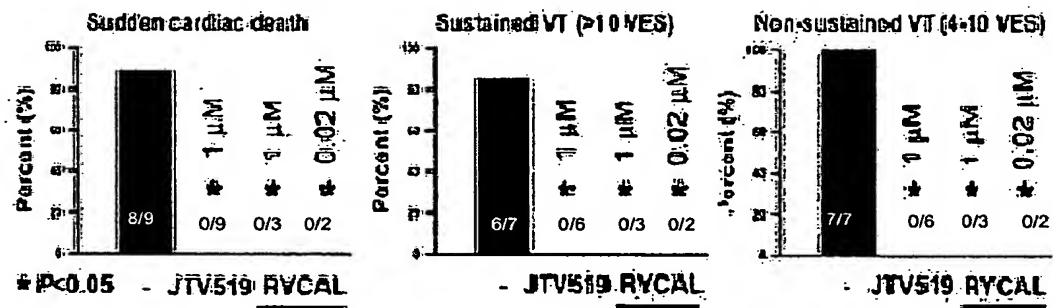


FIG. 4D

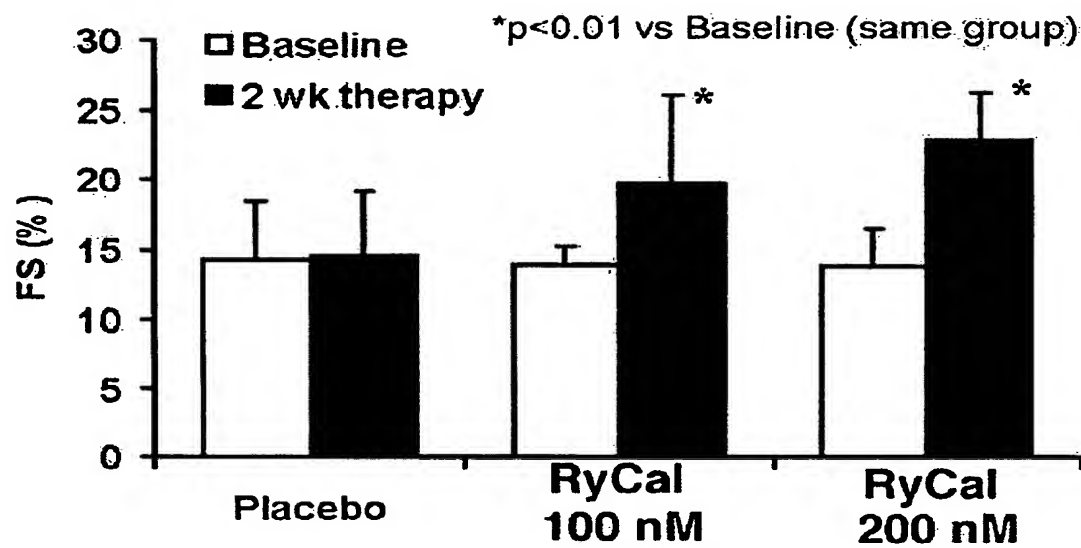


FIG. 5

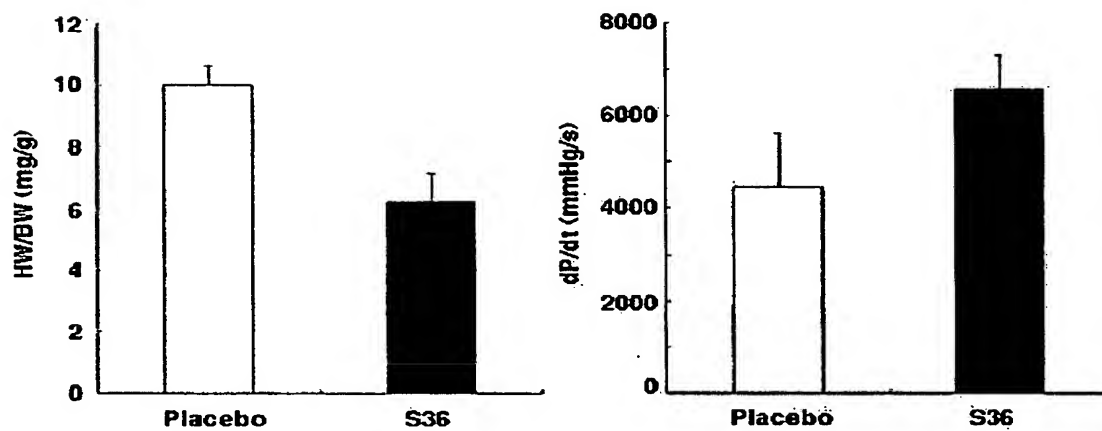


FIG. 6

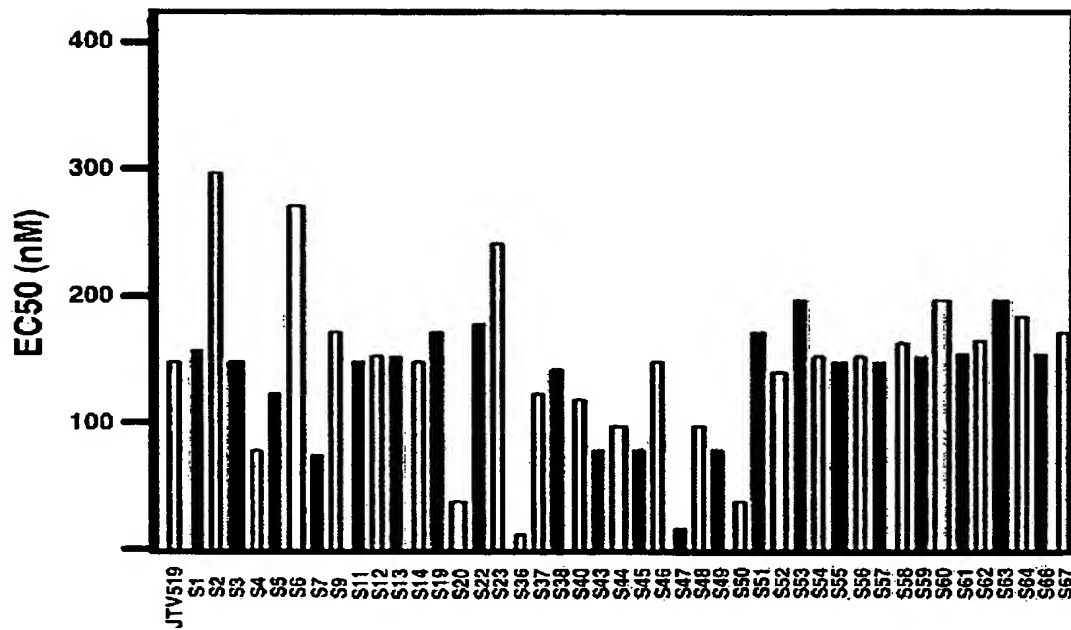


FIG. 7

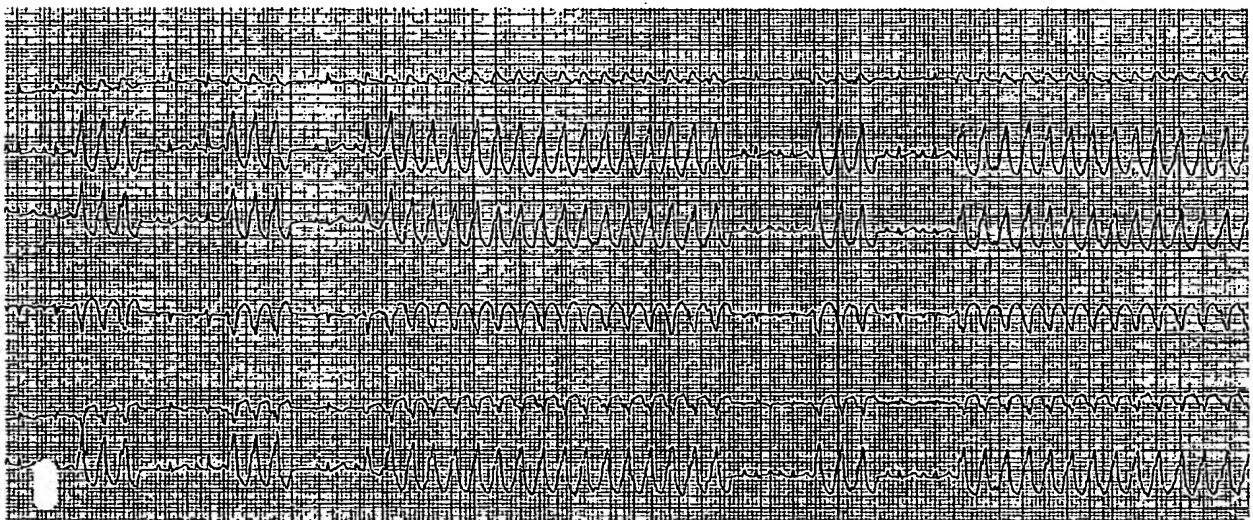


FIG. 8



FIG. 9